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Description

Technical Fields

This invention is in the fields of molecular biology and virology and in particular relates to human T cell leukemia virus - type III (HTLV-III).

Background

The term human T cell leukemia-lymphoma virus (HTLV) refers to a unique family of T cell tropic retroviruses. These viruses play an important role in the pathogenesis of certain T cell neoplasms. There are presently three known types of HTLVs. One subgroup of the family, HTLV-type I (HTLV-I), is linked to the cause of adult T-cell leukemia-lymphoma (ATLL) that occurs in certain regions of Japan, the Caribbean and Africa. HTLV-type II (HTLV-II) has been isolated from a patient with a T-cell variant of hairy cell leukemia. M.Popovic et al., Detection, Isolation, and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and Pre-AIDS. Science, 224:497-500 (1984).

HTLV-type III (HTLV-III) has been isolated from many patients with acquired immunodeficiency syndrome (AIDS). HTLV-III refers to prototype virus isolated from AIDS patients. Groups reported to be at greatest risk for AIDS include homosexual or bisexual males; intravenous drug users and Haitian immigrants to the United States. Hemophiliacs who receive blood products pooled from donors and recipients of multiple blood transfusions are also at risk. Clinical manifestations of AIDS include severe, unexplained immune deficiency which generally involves a depletion of helper T lymphocytes. These may be accompanied by malignancies and infections. The mortality rate for patients with AIDS is high. A less severe form of AIDS also exists, in which there may be lymphadenopathy and depressed helper T cell counts; there is not, however, the devastating illness characteristic of full-blown AIDS. There are many individuals, who are classified as having early AIDS (pre-AIDS), who exhibit these signs. It is not now possible to predict who among then will develop the more serious symptoms.

Much of the evidence implicates HTLV-III as the etiological agent of the infectious AIDS. First, there is consistent epidemiology; greater than 95% of the patients with AIDS have antibodies specific for HTLV-III. Second, there has been reproducible identification and isolation of virus in this disease; more than 100 variants of HTLV-III have been isolated from AIDS patients. Third, there has been transmission of the disease to normal healthy individuals who received blood transfusions from infected blood donors.

HTLV-III has been shown to share several properties with HTLV-I and HTLV-II but also to be morphologically, biologically and antigenically distinguishable. R.C. Gallo et al., Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and At Risk for AIDS. Science, 224:500-503. (1984). For example, HTLV-III has been shown to be antigenically related to HTLV-I and HTLV-II by demonstrating cross-reactivity with antibodies to HTLV-I and HTLV-II core proteins, p24 and p19, and envelope antigens and by nucleic acid cross-hybridization studies with cloned HTLV-I and HTLV-II DNAs. However, unlike HTLV-I and HTLV-II, it lacked the ability to infect and transform T cells from normal umbilical cord blood and bone marrow in vitro, and has the cytopathic effect on infected cells only.

Like the RNA genome of other retroviruses, the RNA genome of HTLV-III contains three genes which encode viral proteins: 1) the gag gene, which encodes the internal structural (nucleocapsid or core) proteins; 2) the pol gene, which encodes the RNA-directed DNA polymerase (reverse transcriptase); and 3) the env gene, which encodes the envelope glycoproteins of the virion. In addition, the HTLV-III genome contains a region designated Px, located between the env gene and the 3' LTR, which appears to be involved in functional killing of the virus.

At this time, AIDS is still difficult to diagnose before the onset of clinical manifestations. There is no method presently available for the prevention of the disease. Treatment of those with AIDS is generally not successful and victims succumb to the devastating effects HTLV-III has on the body.

Summary of the Invention

This invention is based upon applicant's cloning of HTLV-III DNA in recombinant/vector host systems capable of expressing immunoreactive HTLV-III polypeptides. Based on the cloning of HTLV-III DNA in systems which express immunoreactive-polypeptides, applicant has developed methods useful in the diagnosis, treatment and prevention of AIDS. Applicant has developed methods of detecting HTLV-III and antibodies against HTLV-III in body fluids (e.g., blood, saliva, semen), and methods useful in immunotherapy (e.g., vaccination and passive immunization against AIDS). In addition, applicant has developed methods of making HTLV-III DNA probes and RNA probes useful in detecting HTLV-III in body fluids.

Polypeptides encoded by a segment of the HTLV-III genome have been produced by these recombinant DNA methods. The polypeptides encoded by a 1.1Kb EcoRI restriction fragment from

HTLV-III cDNA have been produced. The polypeptides expressed have been isolated. These polypeptides are immunoreactive with sera of patients having AIDS and with antibodies to HTLV-III and thus are useful in screening blood and other body fluids for the presence of antibodies against HTLV-III. Applicant's invention therefore provides a method not only for diagnosing AIDS, but also for preventing the transmission of the disease to others through blood or blood components harboring HTLV-III. The latter is particularly valuable in screening donated blood before it is transfused or used to obtain blood components (e.g., Factor VIII for the treatment of hemophilia; Factor IX)
methods are employed in the production of antibodies, including monoclonal antibodies, against the virus. Such antibodies form the basis for immunoassay and diagnostic techniques for directly detecting HTLV-III in body fluids such as blood, saliva, semen, etc. Neutralizing antibodies against the virus may be used to passively immunize against the disease.

Applicant's cloning of HTLV-III DNA in such recombinant vector host systems also provides the basis for determination of the nucleotide sequence HTLV-III DNA. The DNA probes are essentially homologous to the 1.1 Kb EcoRI DNA which is unique to the HTLV-III genome. DNA probes provide another method of detecting HTLV-III in blood, saliva or other body fluids. RNA probes which contain regions unique to the HTLV-III genome can also be formed and used for the detection of HTLV-III in body fluids.

Brief Description of the Figures

Figure 1 is a representation of HTLV-III DNA. Figure 1a shows sites at which the genome is cut by the restriction enzyme SstI and Figure 1b shows the fragments of HTLV-III genome produced through the action of restriction enzymes Kpn, EcoRI and Hind III.

Figure 2 is a representation of HTLV-III DNA. Figure 2a shows the location of restriction enzyme sites in the genome and Figure 2b shows the location in the HTLV-III genome of DNA inserts in open reading frame clones. The (+) and (-) indicate reactivity and lack of reactivity, respectively, of the fusion protein expressed by cells transformed by the ORF vectors with sera of AIDS patients.

Figure 3 shows the nucleotide sequence for HTLV-III DNA and the predicted amino acid sequence of the four longest open reading frames. Restriction enzyme sites are indicated above the nucleotide sequence.

Figure 4 is an immunoblot showing the position on an SDS polyacrylamide gel of HTLV-III env-Beta-galactosidase fusion proteins.

Figure 5 shows sites at which the genome is cut by the restriction enzyme EcoRI and construction of recombinant plasmids carrying HTLV-III DNA.

Figure 6 is an immunoblot showing the positions on nitrocellulose blots of peptides produced by bacterial cells transformed by recombinant constructs ompA1-R-6; ompA2-R-7 and ompA3-R-3, into which a 1.1Kb EcoRI HTLV-III cDNA restriction fragment had been inserted. Figure 6a shows the nucleotide sequence of the ompA signal peptide and the pertinent region of recombinant plasmids ompA1-R-6; ompA2-R-7 and ompA3-R-3.

Figure 7 is an immunoblot showing blocking of reaction between HTLV-III antigens and an AIDS serum by lysates of E.coli containing HTLV-III DNA recombinant plasmid ompA1-R-6 (lanes 1-5) and no blocking of the reaction by lysates of E.coli control cells (lanes 6-10).

Figure 8 is an immunoblot showing the presence or absence of antibodies against the peptide encoded by the 1.1Kb EcoRI HTLV-III restriction fragment of HTLV-III cDNA in sera from healthy individuals (lanes 1-3) and from AIDS patients (lanes 4-11). Purified HTLV-III virus (panel A) or total cell lysate of bacterial clone ompA1-R-6-(O1R6) were reacted with sera samples.

Figure 9 represents the open reading frame expression vector pMRIOO having HTLV-III DNA.

Figure 10 represents lambdaCI-HTLV-III beta-galactosidase fusion proteins. Figure 10a is an immunoblot showing the position on SDS polyacrylamide gel of lambdaCI-HTLV-III beta-galactosidase fusion proteins, and Figure 10b shows the immunoreactivity of such proteins with sera from AIDS patients.

Best Mode of Carrying Out the Invention

Despite the similarity between HTLV-III and the other members of the HTLV-bovine leukemia virus (BLV) family of viruses, the biology and pathology of HTLV-III differs substantially. For example, relatively little homology has been found in the HTLV-III genome when compared with that of the HTLV-I or -II genome. Infection with HTLV-III often results in profound immunosuppression (AIDS), consequent to the depletion of the OKT4(+) cell population. This effect is mirrored by a pronounced cytopathic, rather than transforming, effect of HTLV-III infection upon the OKT4(+) cells in lymphocyte cultures in vitro. In contrast, infection with HTLV-I results in a low incidence of T-cell leukemia lymphoma (an OKT4(+) cell malignancy). There is evidence for some degree of immunodeficiency in HTLV-I patients as well. Infection of primary lymphocytes in culture by HTLV-I and -II results in

vitro transformation of predominantly OKT4(+) cells. A cytopathic effect of HTLV-I infection upon lymphocytes is apparent, but the effect is not as pronounced as that observed for HTLV-III.

HTLV-III also differs from HTLV-I and -II in the extent of infectious virion production in vivo and in vitro. High titers of cell free, infectious virions can be obtained from AIDS patient semen and saliva and from the supernatant of cultures infected with HTLV-III. Very few, if any, cell free infectious virions can be recovered from adult T-cell leukemia lymphoma (ATLL) patients or from cultures infected with HTLV-I or -II.

Envelope glycoprotein is the major antigen recognized by the antiserum of AIDS patients. In this respect, HTLV resembles other retroviruses, for which the envelope glycoprotein is typically the most antigenic viral polypeptide. In addition, the neutralizing antibodies are generally directed toward the envelope glycoprotein of the retrovirus. Serum samples from 88 percent to 100 percent of those with AIDS have been shown to have antibodies reactive with antigens of HTLV-III; the major immune reactivity was directed against p41, the presumed envelope antigen of HTLV-III. Antibodies to core proteins have also been demonstrated in serum of AIDS patients, but do not appear to be as effective an indicator of infection as is the presence of antibodies to envelope antigen.

The p41 antigen of HTLV-III has been difficult to characterize because the viral envelope is partially destroyed during the process of virus inactivation and purification. This invention responds to the great need to characterize this antigenic component of the HTLV-III virus and to determine the existence and identity of other viral antigenic components in several ways. It provides products, such as HTLV-III polypeptides, antibodies to the polypeptides and RNA and DNA probes, as well as methods for their production. These serve as the basis for screening, diagnostic and therapeutic products and methods.

This invention relates to HTLV-III polypeptides which are produced by translation of a recombinant DNA sequence encoding HTLV-III proteins. Polypeptides which are produced in this way and which are immunoreactive with serum from AIDS patients or antibodies to HTLV-III are referred to as recombinant DNA-produced immunoreactive HTLV-III polypeptides. They include the polypeptides which are produced by the translation of the recombinant DNA sequences included in a 1.1Kb EcoRI restriction fragment of HTLV-III cDNA.

The polypeptides encoded by this region of the HTLV III can be used in immunochemical assays for detecting antibodies against HTLV-III and HTLV-VIII infection. These methods can assist in diagnosing AIDS. In addition, they can also be employed to screen blood before it is used for transfusions or for the production of blood components (e.g., Factor VIII for the treatment of hemophilia). Availability of screening technics will reduce the risk of AIDS transmission.

Detection of antibodies reactive with the polypeptides can be carried out by a number of established methods. For example, an immunoreactive HTLV III polypeptide can be affixed to a solid phase (such as polystyrene bead or other solid support). The sold phase is then incubated with blood sample to be tested for antibody against HTLV-III. After an appropriate incubation period the solid phase and blood sample are separated. Antibody bound to the solid phase can be detected with labeled polypeptide or with a labeled antibody against human immunoglobulin.

The HTLV-III polypeptides can be used in a vaccine prevention of AIDS.

The polypeptides can also be used to produce antibodies, including monoclonal antibodies, against the HTLV-III polypeptides. These antibodies can be used in immunochemical assays for direct detection of the virus in body fluids (such as blood, saliva and semen). Assays employing monoclonal antibody against specific HTLV III antigenic determinants will reduce false-positive results thereby improving accuracy of assays for the virus. Antibodies against the virus may also be useful in immuno-therapy. For example, antibodies may be used to passively immunize against the virus. The methods of producing the polypeptides are also a subject of this invention, as are diagnostic methods based on these polypeptides.

HTLV-III POLYPEPTIDES

Genetic engineering methods are used to isolate a 15Kd peptide encoded by a 1.1Kb EcoRI HTLV-III restriction fragment of HTLV-III DNA. These methods are also used to sequence the fragments which encode the polypeptides. The proviral genes integrated into host cell DNA are molecularly cloned and the nucleotide sequences of the cloned provirus is determined.

An E. coli expression library of HTLV-III DNA is constructed. The HTLV-III genome is cloned and cuts are then made in the cloned HTLV-III genome with restriction enzymes to produce DNA fragments. (Figures 1 and 2) HTLV-III DNA fragments of approximately 200-500bp are isolated from agarose gel, end repaired with T_4 polymerase and ligated to linker DNA. The linker ligated DNA is then treated with a restriction enzyme, purified from agarose gel and cloned in an expression vector. Examples of the expression vectors used are: OmpA, pIN (A,B and C), lambda pL, T7, lac, Trp, ORF and lambda gt11. In addition, mammalian

cell vectors such as pSV28pt, pSV2neo, pSVdhfr and VPV vectors, and yeast vectors, such as GALI and GAL10, may be used.

The bacterial vectors contain the lac coding sequences, into which HTLV-III DNA can be inserted for the generation of B-galactosidase fusion protein. The recombinant vectors are then introduced into bacteria (e.g., E.coli); those cells which take up a vector containing HTLV-III DNA are said to be transformed. The cells are then screened to identify cells which have been transformed and are expressing the fusion protein. For example, the bacteria are plated on MacConkey agar plates in order to verify the phenotype of clone. If functional B-galoctosidase is being produced, the colony will appear red.

Bacterial colonies are also screened with HTLV-III DNA probes to identify clones containing the DNA region of interest. Clones which are positive when screened with the DNA probe and positive on the MacConkey agar plates are isolated.

This identification of cells harboring the HTLV-III DNA sequences makes it possible to produce HTLV-III polypeptides which are immunoreactive with HTLV-III specific antibody. The cells from the selected colonies are grown in culture under conditions allowing the expression of the hybrid protein. Cell protein is then obtained by means known in the art. For example, the culture can be centrifuged and the resulting cell pellet broken. Polypeptides secreted by the host cell can be obtained (without disruption of the cells) from the cell culture supernatant.

The total cellular protein is analysed by being run on an SDS polyacrylamide gel electrophoresis. The fusion proteins are identified at a position on the gel which contains no other protein. Western blot analyses are also carried out on the clones which screened positive. Such analyses are performed with serum from AIDS patients, with the result that it is possible to identify those clones expressing HTLV-III B-galactosidase fusion proteins (antigens) that cross-react with the HTLV-III specific antibody.

Lambda $_{10}$ clones harboring HTLV-III DNA are cloned from the replicated form of the virus. As the retrovirus is replicating, double stranded DNA is being produced. The cloned HTLV-III DNA is digested with the restriction enzyme SstI. (Figure 1a) Because there are two SstI recognition sites within the LTR of HTLV-III DNA, one LTR region is not present in the cloned DNA sequence removed from the lambda$_{10}$ vector. As a result, a small (approximately 200 bp) fragment of the HTLV-III DNA is missing.

The resulting DNA is linearized and fragments are produced by digesting the linearized genomic DNA spanning the env gene region with the restriction enzyme EcoRI (Figure 1b). The resulting 1.1KbEcoRI-EcoRI fragments are isolated by gel electrophoresis and electroelution. These fragments are randomly sheared to produce smaller fragments. The fragments thus produced are separatedfrom agarose gel and DNA fragments between about 200-500 bp are eluted.

The eluted 200-500bp DNA fragments are end filled through the use of E. coli T_4 polymerase and blunt end ligated into an open reading frame expression (ORF) vector, such as pMR100. This ligation may occur at the SmaI site of the pMR100 vector, which contains two promoter regions, hybrid coding sequences of lambdaCI gene and lacI-LacZ gene fusion sequence. In the vector, these are out of frame sequences; as a result, the vector is nonproductive. The HTLV-III DNA is inserted into the vector; the correct DNA fragments will correct the reading frame, with the result that CI-HTLV-III-B-galactosidase fusion proteins are produced. The expression of the hybrid is under the control of the lac promoter. Based on the sequence of pMR100, it appears that if a DNA fragment insert cloned into the SmaI site is to generate a proper open reading frame between the lambdaCI gene fragment and the lac-Z fragment, the inserted DNA must not contain any stop codons in the reading frame set by the frame of the lambdaCI gene.

The recombinant pMR100 vectors are then introduced into E. coli. The bacteria are plated on MacConkey agar plates to verify the phenotype of the clone. If functional B-galactosidase is being produced, the colony will appear red. The colonies are also screened with HTLV-III DNA probes, for the purpose of identifying those clones containing the insert. Clones which are positive when screened with the DNA probe and positive on the MacConkey agar plates are isolated.

The cells from the selected colonies are grown in culture. The culture is spun down and the cell pellet broken. Total cellular protein is analysed by being run on an SDS polyacrylamide gel. The fusion proteins are identified at a position on the gel which contains no other protein. (Figure 4)

Western blot analyses are also carried out on the clones which screened positive. Sera from AIDS patients are used, thus making it possible to identify those clones which express the HTLV-III-B-galactosidase fusion proteins that cross-react with the HTLV-III specific antibody. 1000 clones were screened by this method; 6 were positive.

Because of the nature of the pMR100 cloning vehicle, a productive DNA insert should also be expressed as a part of a larger fusion polypeptide. HTLV-III env gene containing recombinant clones was identified by colony hybridization. The production of larger fusion polypeptides bearing functional B-galactosidase activity was verified by phenotype

identification on MacConkey agar plates; by B-galactosidase enzymatic assays and by analysis on 75% SDS-polyacrylamide gels. Immunoreactivity of the larger protein with antibody to HTLV-III was assessed by western blot analysis using serum from AIDS patients. These large fusion proteins also reacted with anti-B-galactosidase and anti-CI antiserum. This finding is consistent with the hypothesis that they are proteins of CI-HTLV-III-lacIZ.

The open reading frame insert fragment of HTLV-III is further analyzed by DNA sequencing analysis. Because one of the two BamHI sites flanking the SmaI cloning site in pMR100 is destroyed in the cloning step, positive clones are digested with restriction enzymes HindIII and claI to liberate the inserted HTLV-III DNA fragment. The HTLV-III ORF inserts are isolated from the fusion recombinant and cloned into M13 sequencing cloning vector mp18 and mp19 digested with HindIII and AccI. DNA sequences of the positive ORF clones are then determined.

Fragments of HTLV-III DNA of approximately 200-500 bps are isolated from agarose gel, end repaired with T_4 polymerase and ligated to EcoRI linker. The EcoRI linker ligated DNA is then treated with EcoRI purified from 1% agarose gel and cloned in an expression vector, lambda gt11. This vector contains lac Z gene coding sequences into which the foreign DNA can be inserted for the generation of B-galactosidase fusion protein. The expression of the hybrid gene is under the control of lac repressor. The lac repressor gene, lac I, is carried on a separate plasmid pMC9 in the host cell, E. coli Y1090. AIDS patient serum was used to probe the lambdagt11 library of HTLV-III genome DNA containing $1.5x10^4$ recombinant phage. In a screen of 5000 recombinants, 100 independent clones that produced strong signals were isolated. The positive recombinant DNA clones were further characterized for their specific gene expression. Rabbit hyperimmune serum against P24 was also used to identify the gag gene specific clones. Nick-translated DNA probes of specific HTLV-III gene, specifically the gag gene, env gene and Px gene were used to group the positive immunoreactive clones into specific gene region.

Recombinant clones that produced strong signals with AIDS serum and contain insert DNA spanning the HTLV-III gag, pol, sor and env-lor gene regions were examined in detail by mapping their insert with restriction enzymes and DNA sequencing analysis.

Determination of the Nucleotide Sequence of HTLV-III DNA

Genetic engineering methods are used to determine the nucleotide sequence of HTLV-III DNA. One technique that can be used to determine the sequence is a shotgun/random sequencing methods. HTLV-III DNA is sheared randomly into fragments of about 300-500 bp in size. The fragments are cloned, for example, using m13, and the colonies screened to identify those having an HTLV-III DNA fragment insert. The nucleotide sequence is then generated, with multiple analysis producing overlaps in the sequence. Both strands of the HTLV-III DNA are sequenced to determine orientation. Restriction mapping is used to check the sequencing data generated.

The nucleotide sequence of one cloned HTLV-III genome (BH10) is shown in Figure 3, in which the position of sequences encoding gag protein p17 and the N-terminus of gag p24 and the C-terminus of gag p15 (which overlaps with the N-terminus of the pol protein) are indicated. The open reading frames (ORF) for pol, sor and env-lor are also indicated. The sequence of the remaining 182 base pairs of the HTLV-III DNA not present in clone BH10 (including a portion of R, U5, the tRNA primer binding site and a portion of the leader sequence) was derived from clone HXB2. The sequences of two additional clones (BH8 and BH5) are also shown. Restriction enzyme sites are listed above the nucleotide sequence; sites present in clone BH8 but not in clone BH10 are in parentheses. Deletions are noted ([]) at nucleotides 251, 254, 5671 and 6987-7001. The nucleotide positions (to the right of each line) start with the transcriptional initiation site. The amino acid residues are numbered (to the right of each line) for the four largest open reading frames starting after the preceding termination codon in each case except gag which is enumerated from the first methionine codon. A proposed peptide cleavage site (V) and possible asparagine-linked glycosylation sites are shown (*) for the env-lor open reading frame. The sequences in the LTR derived from clones BH8 and BH10 listed in the beginning of the figure are derived from the 3'-portion of each clone and are assumed to be identical to those present in the 5'-LTR of the integrated copies of these viral genomes.

Clone HXB2 was derived from a recombinant phage library of XbaI digested DNA from HTLV-III infected H9 cells cloned in lambdaJ1. H9 cells are human leukemic cells infected by a pool of HTLV-III from blood of AIDS patients, F. Wong-Staal, Nature, 312, November, 1984. Cloning vector clones BH10, BH8, and BH5 were derived from a library of SstI digested DNA from the Hirt supernatant fraction of HTLV-III infected H9 cells cloned in lambdagtWes.lambdaB. Both libraries were screened with cDNA probe synthesized from virion RNA using oligo.dT as a primer. Clones BH8, BH5, and a portion of HXB2 were sequenced as de-

scribed by Maxam and Gilbert. (1980) Maxam, A. M. and Gilbert, Co. Methods in Enzymology. 65: 499-560. Clone BH10 was sequenced by the method of Sanger modified by the use of oligonucleotides complementary to the M13 insert sequence as primers and using Klenow fragment of DNA polymerase I or reverse transcriptase as the polymerase.

Formation of RNA, RNA Probes and DNA Probes Specific to HTLV-III

DNA sequences which are an entire gene or segment of a gene from HTLV-III are inserted into a vector, such as a T7 vector. In this embodiment, the vector has the Tceu promoter from the T cell gene 10 promoter and DNA sequences encoding eleven amino acids from the T cell gene 10 protein.

The vectors are then used to transform cells, such as E. coli. The T7 vector makes use of the T7 polymerase, which catalyzes RNA formation and recognizes only T7 promoter, which is the site where RNA polymerase binds for the initiation of transcription. The T7 polymerase does not recognize E. coli promoter. As a result, if HTLV-III DNA sequences are inserted after the promoter and polymerase genes of the T7 vector, which recognizes them to the exclusion of other signals, and a terminator is placed immediately after the HTLV-III DNA sequences, the T7 vector will direct manufacture RNA complementary to the HTLV-III DNA insert.

Determination of the nucleotide sequence of HTLV-III DNA also provides the basis for the formation of DNA probes. Both RNA probes and DNA HTLV-III probes must have the distinctive region of the HTLV-III genome in order to be useful in detecting HTLV-III in body fluids. There is relatively little homology between the HTLV-III genome and the HTLV-I and -II genomes and probes contain regions which are unique to HTLV-III (i.e., not shared with HTLV-I or -II).

Either viral RNA or DNA can be used for detecting HTLV-III in, for example, saliva, which is known to have a very high concentration of the virus. This can be done, for example, by means of a dot blot, in which the saliva sample is denatured, blotted onto paper and then screened using either type of probe. If saliva is used as the test fluid, detection of HTLV-III is considerable faster and easier than is the case if blood is tested.

Production of Monoclonal Antibodies Reactive with HTLV-III Polypeptides

Monoclonal antibodies reactive with HTLV-III polypeptides are produced by antibody-producing cell lines. The antibody-producing cell lines may be hybridoma cell lines commonly known as hybridomas. The hybrid cells are formed by fusion of cells which produce antibody to HTLV-III polypeptide and an immortalizing cell, that is, a cell which imparts long term tissue culture stability on the hybrid cell. In the formation of the hybrid cell lines, the first fusion partner - the antibody-producing cell - can be a spleen cell of an animal immunized against HTLV-III polypeptide. Alternatively, the antibody-producing cell can be isolated B lymphocyte which produces antibody against an HTLV-III antigen. The lymphocyte can be obtained from the spleen, peripheral blood, lymph nodes or other tissue. The second fusion partner - the immortal cell - can be a lymphoblastoid cell or a plasmacytoma cell such as a myeloma cell, itself an antibody-producing cell but also malignant.

Murine hybridomas which produce monoclonal antibodies against HTLV-III polypeptide are formed by the fusion of mouse myeloma cells and spleen cells from mice immunized against the polypeptide. To immunize the mice, a variety of different immunization protocols may be followed. For instance mice may receive primary and boosting immunizations of the purified polypeptide. The fusions are accomplished by standard procedures. Kohler and Milstein, (1975) Nature (London) 256, 495-497; Kennet, R., (1980) in Monoclonal Antibodies - (Kennet et al., Eds. pp. 365-367, Plenum Press, NY).

The hybridomas are then screened for production of antibody reactive with the polypeptide. This can be performed by screening procedures known in the art.

Another way of forming the antibody-producing cell line is by transformation of antibody-producing cells. For example, a B lymphocyte obtained from an animal immunized against HTLV-III polypeptide may be infected and transformeed with a virus such as the Epstein-Barr virus in the case of human B lymphocytes to give an immortal antibody-producing cell. See, e.g., Kozbor and Rodor (1983) Immunology Today 4(3), 72-79. Alternatively, the B lymphocyte may be transformed by a transforming gene or transforming gene product.

The monoclonal antibodies against HTLV-III polypeptide can be produced in large quantities by injecting antibody-producing hybridomas into the peritoneal cavity of mice and, after an appropriate time, harvesting the ascites fluid which contains very high titer of homogenous antibody and isolating the monoclonal antibodies therefrom. Xenogeneic hybridomas should be injected into irradiated or athymic nude mice. Alternatively, the antibodies may be produced by culturing cells which produce HTLV-III polypeptide in vitro and isolating secreted monoclonal antibodies from the cell cul-

ture medium. The antibodies produced according to these methods can be used in diagnostic assays (e.g., detecting HTLV-III in body fluids) and in passive immunotherapy. The antibodies reactive with HTLV-III polypeptides provide the basis for diagnostic tests for the detection of AIDS or the presence of HTLV-III in biological fluids (e.g., blood, semen, saliva) and for passive immunotherapy. For example, it is possible to produce anti p 41, to attach it to a solid phase using conventional techniques and to contact the body fluid to be tested with the immobilized antibody. In this way, HTLV-III (antigen) can be detected in the body fluid; this method results in far fewer false positive test results than do tests, in which antibody against HTLV-VIII is detected.

This invention will now be further illustrated by the following examples.

EXAMPLE 1

PREPARATION OF SONICATED DNA FRAGMENTS

10 ug of gel purified HTLV-III restriction fragments were sonicated to fragment size on average of 500 bps. After sonication, the DNA was passed through a DEAE-cellulose column in 0.1XTBE in order to reduce the volume. The DEAE-bound DNA was washed with 5 ml of 0.2 M NaCl-TE (2 M NaCl, 10 mm Tris HCl pH 7.5, 1 mM EDTA) and then eluted with 1 M NaCl-TE, and ethanol precipitated. The size range of the sonicated DNA was then determined on 1.2% agarose gel. DNA fragments of desired length (200-500 bps) was eluted from the gel. T4 DNA polymerase was used to fill in and/or trim the single strand DNA termini generated by the sonication procedure. DNA fragments were incubated with T4 polymerase in the absence of added nucleotides for five minutes at 37 • C to remove nucleotides from 3' end and then all 4 nucleotide precursors were added to a final concentration of 100 uM and the reaction mixture was incubated another 30 minutes to repair the 5'-end single stranded overhang. The reaction was stopped by heat inactivation of the enzyme at 68 • C for 10 minutes. DNA was phenol extracted once, ethanol precipitated and resuspended in TE.

EXAMPLE 2

CLONING OF RANDOM SHEARED DNA FRAGMENTS

The sonicated blunt end repaired HTLV-III DNA fragments were ligated into the SmaI site of the ORF expression vector pMR100 and transformed into host cell LG90 using standard transformation procedures. B-galactosidase positive phenotype of the transformant were identified by plating the transformed cell on ampicillin (25 ug/ml) containing McConkey agar plates and scoring the phenotype after 20 hours at 37 • C.

EXAMPLE 3

HYBRID PROTEIN ANALYSIS

Ten milliliter samples of cells from an overnight saturated culture grown in L broth containing ampicillin (25 ug/ml) were centrifuged, the cell pellet was resuspended in 500 ul of 1.2 fold concentrated Laemmli sample buffer. The cells were resuspended by vortexing and boiling for 3 minutes at 100 • C. The lysate was then repeated by being forced through a 22 guage needle to reduce the lysate viscosity. Approximately 10 ul of the protein samples were electrophoresed in 7.5% SDS-PAGE (SDS-polyacrylamide) gels.

Electrophoretic transfer of proteins from SDS-PAGE gels to nitrocellulose paper was carried out according to Towbin et. al. Proc. Natl. Acad. Sci. USA, 76, 1979, 4350-54. After the transfer, the filter was incubated at 37 • C for two hours in a solution of 5% (w/v) nonfat milk in PBS containing 0.1% antifoam A and 0.0001% merthiolate to saturate all available protein binding sites. Reactions with AIDS antisera were carried out in the same milk buffer containing 1% AIDS patient antisera that had been preabsorbed with E. coli lysate. Reactions were performed in a sealed plastic bag at 4 • C for 18-24 hours on a rotatory shaker. Following this incubation, the filter was washed three times for 20 minutes each at room temperature in a solution containing 0.5% deoxycholic, 0.1 M NaCl, 0.5% triton X-100, 10 mm phosphate buffer pH 7.5 and 0.1 mM PMSF.

To visualize antigen-antibody interactions, the nitrocellulose was then incubated with the second goat antihuman antibody that had been iodinated with ^{125}I. The reaction with the iodinated antibody was carried out at room temperature for 30 minutes in the same milk buffer as was used for the first antibody. The nitrocellulose was then washed as previously described and exposed at -70 • C using Kodak XAR5 film with an intensifying screen.

EXAMPLE 4

SCREENING OF THE HTLV-III ORF LIBRARY BY COLONY HYBRIDIZATION

E. coli LG90 transformants were screened with HTLV-III DNA probes containing the DNA regions of interest (e.g. HTLV-III gag, env or Px gene specific sequences). Colonies were grown on ni-

trocellulose filter and screened according to the procedure of Grunstein and Hogness by using a nick-translated HTLV-III DNA as hybridization probe.

The DNA fragment was in general excised by restriction endonuclease digestion, gel purified, and ^{32}P-labeled to a specific activity of $0.5x10^8$ cpm/ug by nick-translation (Rigby, P.W.J. et al., J. Mol. Biol. 113, 237 (1977). Duplicate nitrocellulose filters with DNA fixed to them were prehybridized with 6xSSC (0.9 M NaCl/0.09 M sodium citrate, pH 7.0), 5X Denhardt's solution (Denhardt's solution: 0.02% each of polyvinylpyrrolidone, Ficoll and bovine serum albumin) 10 ug of denatured sonicated E. coli DNA per ml at 55 °C for 3-5 hours. The filters were then placed in a fresh sample of the same solution to which the denatured hybridization probe had been added. Hybridization was permitted to take place at 68 °C for 16 hours. The filters were washed repeatedly in 0.3XSSC at 55 °C, and then exposed to x-ray film.

EXAMPLE 5

RECOMBINANT DNA PRODUCED PEPTIDE OF HTLV-III WHICH IS IMMUNOREACTIVE WITH SERA FROM PATIENTS WITH AIDS

An expression vector, pIN-III-ompA (ompA) was used. ompA has the lipoprotein (the most abundant protein in E.coli) gene promoter (lpp) and the lacUV5 promoter-operator (Figure 5). ompA vectors also contain the DNA segment encoding the lac repressor, which allows the expression of the inserted DNA to be regulated by lac operon inducers such as IPTG. The ompA cloning vehicles contain three unique restriction enzyme sites EcoRI, HindIII, Bam HI in all three reading frames and permit the insertion of DNA into any of these restriction sites.

Various restriction fragments were excised from the recombinant clone, lambdaBH10, which contains a 9 Kb long HTLV-III DNA insert in the SstI site of the vector lambdagtWES lambdaB. These restriction fragments were them inserted into the ompA vectors at all three reading frames and used to transform E.coli JA221 cells. Transformants were first screened for HTLV-III DNA by in situ colony hybridization using nick-translated HTLV-III DNA probes. The positive clones were then screened for expression of HTLV-III antigenic peptides using HTLV-III specific antibodies. For this, lysates of E.coli cell containing HTLV-III DNA recombinant plasmids were electrophoresed on 12.5% SDS-polyacrylamide gel and electroblotted onto nitrocellulose filters The filters were then incubated first with well-characterized sera from AIDS patients and next with ^{125}I-labelled goat anti-human IgG antibodies. The washed filters were autoradiographed to identify peptides reactive with anti-HTLV-III antibodies.

Several gene segments that encode peptides shoring immunoreactivity with anti-HTLV-III antibodies were demonstrated. Among these is a 1.1 Kb EcoRI restriction fragment. This fragment was inserted into ompA vectors in all three reading frames (Figure 5). Cells were grown at 37 °C in L broth containing 100mg/ml. ampicillin to an $^{OD}600$ of 0.2. At this time, the cell cultures were divided into too aliquots. IPTG was added to one aliquot to a final concentration of 2mM (induced). IPTG was not added to the other aliquot (uninduced). Upon IPTG induction, transformants of all three plasmid constructs (designated $OmpA_1$-R-6 (O1R6), O mpA_2-R-7(O2R7), and $OmpA_3$-R-3 (O3R3)) produced a 15 Kd peptide that is strongly reactive with anti-HTLV-III antibodies in sera from AIDS patients (Figure 6 lane 1, purified HTLV-III virions; lanes 2 and 3, O1R6 uninduced and induced; lanes 4 and 5, 02R7 uninduced and induced; lanes 6 and 7 03R3 uninduced and induced). This reactivity is not detected when sera from normal individuals is used.

DNA sequence data of the HTLV-III genome indicates that there is an open reading frame inside the pol gene located at the 5'-end of the EcoRI fragment. DNA sequence analysis of the three recombinant constructs, O1R6, O2R7 and P3R3, confirmed that each of these recombinants has a different reading frame of the HTLV-III plus strand coupled to the coding sequence of each vector. Only in O3R3 is the reading frame of the inserted DNA in phase with that set by the signal peptide in the ompA vector; in O1R6 and O2R7 the pol gene segment DNA is out of phase (Figure 6a).

There is a 6 bp ribosome binding site, AAGGAG (Shine-Dalgarno sequence), located at nucleotide position 24-29 and an initiation codon, ATG, located 11 bp downstream (position 41-43). The 15 Kd peptide synthesized by all three recombinants appears to be translated from the transcripts using this internal initiation codon. If this is true, the peptide starts from the ATG located at position 41-43 and ends at the stop codon at position 446-448, producing a peptide of 135 amino acid residues encoded by the 3'-end segment of the pol gene of HTLV-III.

In addition to the 15 Kd peptide, the O3R3 construct, in which the reading frame of the HTLV-III DNA pol gene is in phase with that set by the vector, produced two additional peptides about 19 Kd and 16.5 Kd in size (Figure 6). It is possible that the 19 Kd peptide contains an additional 35 amino acid residues, 21 of which are from the signal peptide encoded by the $ompA_3$ vector and 14 encoded by the inserted HTLV-III DNA itself. The

16.5 Kd peptide may be the processed 19 Kd peptide in which the signal peptide is cleaved.

The O1R6 and O2R7 constructs also produces another peptide of about 17.5 Kd (Figure 6) and weakly reactive with sera of AIDS patients. The origin of this peptide is not clear. The 1.1 Kb EcoRI fragment contains a second potential coding region designated as the short open reading frame (SOR) extending from nucleotide position 360 to 965 (Figure 5). Four of the five AUG methionine codons in this region are near the 5'-end of this open reading frame. This DNA segment could encode peptides of 192, 185, 177 or 164 amino acid residues. However, there is no clearly recognizable ribosome binding site at the 5'-end of this open reading frame.

Further evidence also supports the conclusion that the 15 Kd peptide is indeed derived from the pol gene. First, deletion of the 3'-end StuI to EcoRI fragment from the 1.1 Kb EcoRI insert from O1R6, 02R7 and O3R8 (Figure 5) does not affect the synthesis of the 15 Kd peptide. Second, clones containing only the 5'-end EcoRI to NdeI fragment still produce the same 15 Kd peptide. Finally, several recombinant clones containing various DNA fragments having the SOR coding sequence properly inserted into the open reading frame cloning vector, pMR100, produced lambdaCI-HTLV-III B-galactosidase tripartite fusion proteins which have very little immunoreactivity with anti-HTLV-III antibodies present in sera from AIDS patients.

Significant immunoreactivity against the 15 Kd peptide derived from the viral pol gene in sera from AIDS patients was detected. The identity of this immunoreactive peptide, with respect to the banding pattern of HTLV-III virion antigen in SDS-polyacrylamide gel electrophoresis, was determined by means of a competition inhibition immunoassay. Purified HTLV-III virions were treated with SDS, electrophoresed, and electroblotted onto a nitrocellulose filter. Identical filter strips containing disrupted HTLV-III virions were incubated with well characterized serum from an AIDS patient in the presence or absence of lysates of O1R6, O2R7, or control bacterial clones. The specific immunoreaction between anti-HTLV-III antibodies present in sera of the AIDS patients and the blotted virion proteins were then revealed by ^{125}I-labeled goat anti-human antibody. As shown in Figure 7, lysates of O1R6 block the immunoreactivity of the viral p31 protein with the AIDS serum, while lysates of control cells do not. This result suggests that the recombinant 15 Kd peptide encoded by 3'-end of the viral pol gene is also a part of another virion protein, p31, in contrast to the view shared by some that p31 is a cellular protein which co-purifies with HTLV-III virions.

The prevalence in the sera of AIDS patients of antibodies against the 15 Kd peptide was also evaluated. In Western blot analysis employing the lysate of O1R6 as the source of antigen, a panel of coded sera from AIDS patients and normal healthy individuals was tested. All of the 20 AIDS sera and none of the 8 normal controls reacted with the 15 Kd peptide. Representative results are shown in (Figure 8). These data indicate that most, if not all, AIDS patients produce antibodies against the viral p31 protein.

Claims

Claims for the following Contracting States : DE, GB, FR, IT, NL, SE, CH, LI, BE, LU

1. HTLV-III polypeptide expressed by cells transformed with a recombinant vector containing HTLV-III DNA, said polypeptide being immunoreactive with sera of individuals with acquired immunodeficiency syndrome or sera containing antibodies to HTLV-III; wherein said HTLV-III DNA is (a) an EcoRI restriction fragment of approximately 1.1 Kb, and having a nucleotide sequence extending from approximately nucleotide 4228 to approximately nucleotide 5327 of the HTLV-III DNA as shown in Figure 3; or (b) an equivalent of said DNA that encodes an immunologically functional equivalent of said polypeptide.

2. An isolated DNA as defined in claim 1(a) or (b) encoding a polypeptide according to claim 1.

3. A recombinant vector containing HTLV-III DNA as defined in claim 1(a) or (b) and capable upon insertion into a host cell, of expressing a polypeptide according to claim 1.

4. pMR 100 vector containing HTLV-III DNA as defined in claim 1(a) or (b) and capable upon insertion into a host cell, of expressing a polypeptide according to claim 1.

5. A hybrid protein comprising a polypeptide according to claim 1 linked to an indicator polypeptide e.g. beta-galactosidase.

6. A DNA probe comprising a DNA sequence essentially homologous to the DNA as defined in claim 1(a) or (b) which encodes a polypeptide according to claim 1.

7. A method of producing a polypeptide according to claim 1, comprising the steps of:
   (a) cleaving HTLV-III DNA to produce DNA fragments;

(b) inserting the DNA fragments into an expression vector to form a recombinant vector;
(c) transforming an appropriate host cell with the recombinant vector; and
(d) culturing the transformed host cell under conditions sufficient for expression of the polypeptide encoded for by the inserted HTLV-III DNA.

8. A method according to claim 7, wherein the cleaving step comprises:
(a) digesting the HTLV-III DNA with restriction endonucleases to produce restriction fragments of DNA, or
(b) shearing the HTLV-III DNA to produce DNA fragments.

9. A method according to claim 7 or claim 8, wherein the expression vector is pMR 100.

10. Monoclonal antibody specifically reactive with a polypeptide according to claim 1.

11. An immunoassay for the detection of HTLV-III employing antibody according to claim 10.

12. A sandwich type immunoradiometric assay for the detection of HTLV-III employing an immobilized antibody according to claim 10, which reacts with HTLV-III polypeptide and a soluble antibody according to claim 10, which reacts with HTLV-III polypeptide.

13. An assay kit comprising an antibody according to claim 10, which reacts specifically with HTLV-III polypeptide bound to a solid phase and a labeled soluble antibody according to claim 10, which reacts specifically with HTLV-III polypeptide.

14. An in vitro method of detecting antibodies against HTLV-III in a bodily fiuld comprising the steps of:
(a) contacting an immunoadsorbent comprising a polypeptide according to claim 1 bound to a solid phase, with a bodily fluid until antibodies against HTLV-III polypeptide in the bodily fluid bind the solid phase polypeptide;
(b) separating the immunoadsorbent from the bodily fluid;
(c) contacting the immunoadsorbent with a labeled polypeptide according to claim 1 or labeled antibody against human immunoglobulin; and
(d) determining the amount of labeled polypeptide bound to immunoadsorbent as an indication of antibody to HTLV-III.

15. A kit for determining the presence of antibody against HTLV-III in a bodily fluid comprising:
(a) an immunoadsorbent comprising HTLV-III polypeptide according to claim 1 bound to a solid phase; and
(b) labeled HTLV-III polypeptide according to claim 1 or a labeled antibody against human immunoglobulin.

16. A method of detecting HTLV-III nucleic acid in a bodily fluid (e.g. a cell lysate) comprising the steps of:
(a) adsorbing the nucleic acid in a bodily fluid onto an adsorbent;
(b) denaturing the adsorbed nucleic acid;
(c) contacting the adsorbed nucleic acid with an HTLV-III DNA probe according to claim 6; and
(d) determining if the probe hybridizes with the adsorbed nucleic acid.

17. A hybridoma cell line which produces antibody specifically reactive with an HTLV-III polypeptide according to claim 1.

18. A polypeptide according to claim 1, for use in therapy e.g. vaccination.

19. Use of monoclonal antibodies according to claim 10, for the manufacture of a medicament for use in immunotherapy against acquired immunodeficiency syndrome.

20. Use of a polypeptide according to claim 1 for the manufacture of a medicament for use in vaccination against acquired immunodeficiency syndrome.

Claims for the following Contracting State : AT

1. A method of producing a polypeptide expressed by cells transformed with a recombinant vector containing HTLV-III DNA, said polypeptide being immunoreactive with sera of individuals with acquired immunodeficiency syndrome or sera containing antibodies to HTLV-III; wherein said HTLV-III DNA is (a) an EcoRI restriction fragment of approximately 1.1 Kb, and having a nucleotide sequence extending from approximately nucleotide 4228 to approximately nucleotide 5327 of the HTLV-III DNA as shown in Figure 3; or (b) an equivalent of said DNA that encodes an immunologically functional equivalent of said polypeptide comprising the steps of:

(i) cleaving HTLV-III DNA to produce DNA fragments;
(ii) inserting the DNA fragments into an expression vector to form a recombinant vector;
(iii) transforming an appropriate host cell with the recombinant vector; and
(iv) culturing the transformed host cell under conditions sufficient for expression of the polypeptide encoded for by the inserted HTLV-III DNA.

2. A method according to claim 1, wherein the cleaving step comprises:
(a) digesting the HTLV-III DNA with restriction endonucleases to produce restriction fragments of DNA, or
(b) shearing the HTLV-III DNA to produce DNA fragments.

3. A method according to claim 1 or claim 2, wherein the expression vector is pMR 100.

4. An immunoassay for the detection of HTLV-III employing an antibody eg. a monoclonal antibody specifically reactive with a polypeptide produced according to the method of claim 1.

5. A sandwich type immunoradiometric assay for the detection of HTLV-III employing an immobilized antibody as defined in claim 4, which reacts with HTLV-III polypeptide and a soluble antibody as defined in claim 4, which reacts with HTLV-III polypeptide.

6. An assay involving the use of a kit comprising an antibody as defined in claim 4, which reacts specifically with HTLV-III polypeptide bound to a solid phase and a labeled soluble antibody as defined in claim 4, which reacts specifically with HTLV-III polypeptide.

7. An in vitro method of detecting antibodies against HTLV-III in a bodily fluid comprising the steps of:
(a) contacting an immunoadsorbent comprising a polypeptide as defined in claim 1 bound to a solid phase, with a bodily fluid until antibodies against HTLV-III polypeptide in the bodily fluid bind the solid phase polypeptide;
(b) separating the immunoadsorbent from the bodily fluid;
(c) contacting the immunoadsorbent with a labeled polypeptide as defined in claim 1 or labeled antibody against human immunoglobulin; and
(d) determining the amount of labeled polypeptide bound to an immunoadsorbent as an indication of antibody to HTLV-III.

8. An assay for determining the presence of antibody against HTLV-III in a bodily fluid involving the use of a kit comprising:
(a) an immunoadsorbent comprising HTLV-III polypeptide as defined in claim 1 bound to a solid phase; and
(b) labeled HTLV-III polypeptide as defined in claim 1 or a labeled antibody against human immunoglobulin.

9. A method of detecting HTLV-III nucleic acid in an bodily fluid (e.g. a cell lysate) comprising the steps of:
(a) adsorbing the nucleic acid in a bodily fluid onto an adsorbent;
(b) denaturing the adsorbed nucleic acid;
(c) contacting the adsorbed nucleic acid with an HTLV-III DNA probe comprising a DNA sequence essentially homologous to the DNA as defined in claim 1(a) or (b);
(d) determining if the probe hybridizes with the adsorbed nucleic acid.

10. Use of monoclonal antibodies as defined in claim 4, for the manufacture of a medicament for use in immunotherapy against acquired immunodeficiency syndrome.

11. Use of a polypeptide as defined in claim 1 for the manufacture of a medicament for use in vaccination against acquired immunodeficiency syndrome.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : DE, GB, FR, IT, NL, SE, CH, LI, BE, LU

1. HTLV-III-Polypeptid, exprimiert von Zellen, die mit einem rekombinanten Vektor transformiert wurden, der HTLV-III DNA enthält, wobei das Polypeptid in bezug auf Seren von Personen mit erworbenem Immundefektsyndrom oder Seren mit Antikörpern gegen HTLV-III immunologisch reaktiv ist; wobei die HTLV-III DNA (a) ein EcoRI Restriktionsfragment von ungefähr 1,1 Kb darstellt und eine Nukleotidsequenz aufweist, die sich ungefähr vom Nukleotid 4228 aus bis ungefähr zum Nukleotid 5327 der HTLV-III DNA gemäß Figur 3 erstreckt; oder (b) ein Äquivalent der genannten DNA darstellt, das für ein immunologisch funktionelles Äquivalent des Polypeptids kodiert.

2. Isolierte DNA gemäß Anspruch 1 (a) oder (b), die für ein Polypeptid nach Anspruch 1 kodiert.

3. Rekombinationsvektor, der HTLV-III DNA gemäß Anspruch 1 (a) oder (b) enthält und nach Insertion in eine Wirtszelle zur Expression eines Polypeptids nach Anspruch 1 in der Lage ist.

4. pMR 100 Vektor, der HTLV-III DNA gemäß Anspruch 1 (a) oder (b) enthält und nach Insertion in eine Wirtszelle zur Expression eines Polypeptids nach Anspruch 1 in der Lage ist.

5. Hybridprotein, das ein Polypeptid gemäß Anspruch 1 enthält, welches mit einem Indikatorpolypeptid wie z. B. Beta-Galactosidase verbunden ist.

6. DNA-Sonde, die eine DNA-Sequenz enthält, welche im wesentlichen homolog zu der für ein Polypeptid nach Anspruch 1 kodierenden DNA gemäß Anspruch 1 (a) oder (b) ist.

7. Verfahren zur Herstellung eines Polypeptids gemäß Anspruch 1, das folgende Schritte aufweist:
   (a) Schneiden der HTLV-III DNA zur Herstellung von DNA-Fragmenten;
   (b) Einfügen der DNA-Fragmente in einen Expressionsvektor zur Herstellung eines Rekombinationsvektors;
   (c) Transformieren einer geeigneten Wirtszelle mit dem Rekombinationsvektor; und
   (d) Kultivieren der transformierten Wirtszelle unter Bedingungen, geeignet zur Expression des Polypeptids, für das die inserierte HTLV-III DNA kodiert.

8. Verfahren nach Anspruch 7, wobei der Schneide-Schritt beinhaltet:
   (a) Schneiden der HTLV-III DNA mittels Restriktionsendonukleasen zur Herstellung von Restriktionsfragmenten der DNA oder
   (b) Scheren der HTLV-III DNA zur Herstellung von DNA-Fragmenten.

9. Verfahren nach Anspruch 7 oder Anspruch 8, wobei der Expressionsvektor pMR 100 ist.

10. Monoklonaler Antikörper, der gegenüber einem Polypeptid gemäß Anspruch 1 spezifisch ist.

11. Immuntest zum Nachweis von HTLV-III, bei dem Antikörper gemäß Anspruch 10 eingesetzt werden.

12. Sandwichartiger immunradiometrischer Test zum Nachweis von HTLV-III, bei dem ein immobilisierter Antikörper gemäß Anspruch 10, der mit HTLV-III-Polypeptid reagiert, und ein löslicher Antikörper gemäß Anspruch 10, der mit HTLV-III-Polypeptid reagiert, eingesetzt werden.

13. Testsystem, enthaltend einen Antikörper gemäß Anspruch 10, der spezifisch mit HTLV-III-Polypeptid reagiert, das an eine feste Phase gebunden ist, sowie einen markierten löslichen Antikörper gemäß Anspruch 10, der spezifisch mit HTLV-III-Polypeptid reagiert.

14. In-vitro-Verfahren zum Nachweis von Antikörpern gegen HTLV-III in Körperflüssigkeit, das folgende Schritte aufweist:
   (a) Kontaktieren eines Immunadsorbens, das Polypeptid gemäß Anspruch 1 aufweist und an eine feste Phase gebunden ist, mit einer Körperflüssigkeit bis Antikörper gegen HTLV-III-Polypeptid in der Körperflüssigkeit an das Polypeptid der festen Phase binden;
   (b) Trennen des Immunadsorbens von der Körperflüssigkeit;
   (c) Kontaktieren des Immunadsorbens mit markiertem Polypeptid gemäß Anspruch 1 oder markiertem Antikörper gegen menschliches Immunglobulin; und
   (d) Bestimmen der Menge des markierten Polypeptids, das an das Immunadsorbens gebunden ist, als Nachweis von Antikörpern gegen HTLV-III.

15. System zum Nachweis des Vorhandenseins von Antikörpern gegen HTLV-III in Körperflüssigkeit enthaltend:
   (a) ein Immunadsorbens, das HTLV-III-Polypeptid gemäß Anspruch 1 enthält, welches an eine feste Phase gebunden ist; und
   (b) markiertes HTLV-III-Polypeptid gemäß Anspruch 1 oder einen markierten Antikörper gegen menschliches Immunglobulin.

16. Verfahren zum Nachweis von HTLV-III-Nukleinsäure in Körperflüssigkeit (z.B. einem Zellysat), das folgende Schritte aufweist:
   (a) Adsorbieren der in Körperflüssigkeit enthaltenen Nukleinsäure auf einem Adsorbens;
   (b) Denaturieren der adsorbierten Nukleinsäure;
   (c) Kontaktieren der adsorbierten Nukleinsäure mit einer HTLV-III DNA-Sonde gemäß Anspruch 6; und
   (d) Nachweisen, ob die Sonde mit der adsorbierten Nukleinsäure hybridisiert.

17. Hybridoma-Zellinie, die Antikörper produziert, welche gegenüber einem HTLV-III-Polypeptid gemäß Anspruch 1 spezifisch sind.

18. Polypeptid gemäß Anspruch 1 zur therapeutischen Verwendung, z. B. zur Impfung.

19. Verwendung monoklonaler Antikörper gemäß Anspruch 10 zur Herstellung eines Arzneimittels zur Verwendung bei der Immuntherapie von erworbenem Immundefektsyndrom.

20. Verwendung eines Polypeptids gemäß Anspruch 1 zur Herstellung eines Arzneimittels zur Verwendung bei der Impfung gegen erworbenes Immundefektsyndrom.

Patentansprüche für folgenden Vertragsstaat : AT

1. Verfahren zur Herstellung eines HTLV-III-Polypeptids, exprimiert von Zellen, die mit einem rekombinanten Vektor transformiert wurden, der HTLV-III DNA enthält, wobei das Polypeptid in bezug auf Seren von Personen mit erworbenem Immundefektsyndrom oder Seren mit Antikörpern gegen HTLV-III immunologisch reaktiv ist; wobei die HTLV-III DNA (a) ein EcoRI Restriktionsfragment von ungefähr 1,1 Kb darstellt und eine Nukleosequenz aufweist, die sich ungefähr vom Nukleotid 4228 aus bis ungefähr zum Nukleotid 5327 der HTLV-III DNA gemäß Figur 3 erstreckt; oder (b) ein Äquivalent der genannten DNA darstellt, das für ein immunologisch funktionelles Äquivalent des Polypeptids kodiert, wobei folgende Schritte vorgesehen sind:
   (i) Schneiden der HTLV-III DNA zur Herstellung von DNA-Fragmenten;
   (ii) Einfügen der DNA-Fragmente in einen Expressionsvektor zur Herstellung eines Rekombinationsvektors;
   (iii) Transformieren einer geeigneten Wirtszelle mit dem Rekombinationsvektor; und
   (iv) Kultivieren der transformierten Wirtszelle unter Bedingungen, geeignet zur Expression des Polypeptids, für das die inserierte HTLV-III DNA kodiert.

2. Verfahren nach Anspruch 1, wobei der Schneide-Schritt beinhaltet:
   (a) Schneiden der HTLV-III DNA mittels Restriktionsendonukleasen zur Herstellung von Restriktionsfragmenten der DNA oder
   (b) Scheren der HTLV-III DNA zur Herstellung von DNA-Fragmenten.

3. Verfahren nach Anspruch 1 oder Anspruch 2, wobei der Expressionsvektor pMR 100 ist.

4. Immuntest zum Nachweis von HTLV-III, bei dem ein Antikörper, wie z.B. ein monoklonaler Antikörper, eingesetzt wird, der spezifisch ist gegenüber einem gemäß dem Verfahren nach Anspruch 1 hergestellten Polypeptid.

5. Sandwichartiger immunradiometrischer Test zum Nachweis von HTLV-III, bei dem ein immobilisierter Antikörper gemäß Anspruch 4, der mit HTLV-III-Polypeptid reagiert, und ein löslicher Antikörper gemäß Anspruch 4, der mit HTLV-III-Polypeptid reagiert, eingesetzt werden.

6. Test unter Verwendung eines Systems, das einen Antikörper gemäß Anspruch 4 enthält, der spezifisch ist gegenüber HTLV-III-Polypeptid, welches an eine feste Phase gebunden ist, und das einen markierten löslichen Antikörper gemäß Anspruch 4 enthält, der spezifisch ist gegenüber HTLV-III-Polypeptid.

7. In-vitro-Verfahren zum Nachweis von Antikörpern gegen HTLV-III in Körperflüssigkeit, das folgende Schritte aufweist:
   (a) Kontaktieren eines Immunadsorbens, das Polypeptid gemäß Anspruch 1 aufweist und an eine feste Phase gebunden ist, mit einer Körperflüssigkeit bis Antikörper gegen HTLV-III-Polypeptid in der Körperflüssigkeit an das Polypeptid der festen Phase binden;
   (b) Trennen des Immunadsorbens von der Körperflüssigkeit;
   (c) Kontaktieren des Immunadsorbens mit markiertem Polypeptid gemäß Anspruch 1 oder markiertem Antikörper gegen menschliches Immunglobulin; und
   (d) Bestimmen der Menge des markierten Polypeptids, das an das Immunadsorbens gebunden ist, als Nachweis von Antikörpern gegen HTLV-III.

8. Test zum Nachweis des Vorhandenseins von Antikörpern gegen HTLV-III in Körperflüssigkeit unter Verwendung eines Systems enthaltend:
   (a) ein Immunadsorbens, das HTLV-III-Polypeptid gemäß Anspruch 1 enthält, welches an eine feste Phase gebunden ist; und
   (b) markiertes HTLV-III-Polypeptid gemäß Anspruch 1 oder einen markierten Antikörper gegen menschliches Immunglobulin.

9. Verfahren zum Nachweis von HTLV-III-Nukleinsäure in Körperflüssigkeit (z.B. einem Zelly-

sat), das folgende Schritte aufweist:
(a) Adsorbieren der in Körperflüssigkeit enthaltenen Nukleinsäure auf einem Adsorbens;
(b) Denaturieren der adsorbierten Nukleinsäure;
(c) Kontaktieren der adsorbierten Nukleinsäure mit einer HTLV-III DNA-Sonde gemäß Anspruch 1 (a) oder (b); und
(d) Nachweisen, ob die Sonde mit der adsorbierten Nukleinsäure hybridisiert.

10. Verwendung monoklonaler Antikörper gemäß Anspruch 4 zur Herstellung eines Arzneimittels zur Verwendung bei der Immuntherapie von erworbenem Immundefektsyndrom.

11. Verwendung eines Polypeptids gemäß Anspruch 1 zur Herstellung eines Arzneimittels zur Verwendung bei der Impfung gegen erworbenes Immundefektsyndrom.

Revendications

Revendications pour les Etats contractants suivants : DE, GB, FR, IT, NL, SE, CH, LI, BE, LU

1. Polypeptide de HTLV-III (virus de la leucémie humaine à lymphocytes T) exprimé par des cellules transformées par un vecteur recombiné contenant l'ADN de HTLV-III, ledit polypeptide étant immunoréactif avec les sérums d'individus atteints du syndrome d'immunodéficience acquise ou avec des sérums contenant des anticorps anti-HTLV-III, ledit ADN de HTLV-III étant (a) un fragment de restriction de EcoRI d'environ 1,1 kilobases, comportant une séquence de nucléotides qui s'étend approximativement du nucléotide 4228 au nucléotide 5327 de l'ADN de HTLV-III tel que représenté sur la fig. 3, ou (b) un équivalent dudit ADN qui code pour un équivalent immunologiquement fonctionnel dudit polypeptide.

2. ADN isolé tel que défini dans la revendication 1(a) ou (b), codant pour un polypeptide selon la revendication 1.

3. Vecteur recombiné, contenant l'ADN de HTLV-III tel que défini dans la revendication 1(a) ou (b) et capable, lorsqu'il est inséré dans une cellule hôte, d'exprimer un polypeptide selon la revendication 1.

4. Vecteur pMR 100, contenant l'ADN de HTLV-III tel que défini dans la revendication 1(a) ou (b) et capable, lorsqu'il est inséré dans une cellule hôte, d'exprimer un polypeptide selon la revendication 1.

5. Protéine hybride, comprenant un polypeptide selon la revendication 1 rattaché a un polypeptide indicateur, par exemple la β-galactosidase.

6. Sonde à ADN, comprenant une séquence d'ADN essentiellement homologue de l'ADN tel que défini dans la revendication 1(a) ou (b), qui code pour un polypeptide selon la revendication 1.

7. Procédé de préparation d'un polypeptide selon la revendication 1, comprenant les étapes consistant:
(a) à couper de l'ADN de HTLV-III pour produire des fragments d'ADN;
(b) à insérer les fragments d'ADN dans un vecteur d'expression pour former un vecteur recombiné;
(c) à transformer une cellule hôte appropriée avec le vecteur recombiné; et
(d) à cultiver la cellule hôte transformée dans des conditions adéquates pour l'expression du polypeptide pour lequel code l'ADN de HTLV-III inséré.

8. Procédé selon la revendication 7, dans lequel l'étape de coupure consiste
(a) à mettre à digérer l'ADN de HTLV-III avec une endonucléase de restriction pour produire des fragments de restriction d'ADN, ou
(b) à couper l'ADN de HTLV-III pour produire des fragments d'ADN.

9. Procédé selon la revendication 7 ou 8, dans lequel le vecteur d'expression est pMR 100.

10. Anticorps monoclonal réagissant spécifiquement avec un polypeptide selon la revendication 1.

11. Essai immunologique pour la détection de HTLV-III, au moyen d'un anticorps selon la revendication 10.

12. Essai immunoradiométrique du type en sandwich pour la détection de HTLV-III, au moyen d'un anticorps immobilisé selon la revendication 10 qui réagit avec le polypeptide de HTLV-III, et d'un anticorps soluble selon la revendication 10 qui réagit avec le polypeptide de HTLV-III.

13. Nécessaire d'essai, comprenant un anticorps selon la revendication 10 qui réagit spécifiquement avec le polypeptide de HTLV-III fixé sur une phase solide, et un anticorps soluble marqué selon la revendication 10 qui réagit spéci-

fiquement avec le polypeptide de HTLV-III.

14. Procédé in vitro de détection d'anticorps anti-HTLV-III dans un fluide corporel, comprenant les étapes consistant
(a) à mettre un immuno-adsorbant, comprenant un polypeptide selon la revendication 1 fixé sur une phase solide, en contact avec un fluide corporel jusqu'a de ce que les anticorps anti-polypeptide de HTLV-III contenus dans le fluide corporel fixent le polypeptide en phase solide;
(b) à séparer l'immuno-adsorbant du fluide corporel;
(c) à mettre l'immuno-adsorbant en contact avec un polypeptide marqué selon la revendication 1 ou un anticorps marqué anti-immunoglobuline humaine; et
(d) à déterminer la quantité de polypeptide marqué qui est fixée à l'immuno-adsorbant, en tant qu'indication de la présence de l'anticorps anti-HTLV-III.

15. Nécessaire pour la détermination de la présence d'anticorps anti-HTLV-III dans un fluide corporel, comprenant:
(a) un immuno-adsorbant comprenant le polypeptide de HTLV-III selon la revendication 1, fixé sur une phase solide; et
(b) le polypeptide de HTLV-III marqué selon la revendication 1 ou un anticorps marqué anti-immunoglobuline humaine.

16. Procédé de détection d'acide nucleique de HTLV-III dans un fluide corporel (par exemple un lysat de cellules), comprenant les étapes consistant:
(a) à adsorber sur un adsorbant l'acide nucléique contenu dans un fluide corporel;
(b) à dénaturer l'acide nucléique adsorbé;
(c) à mettre l'acide nucléique adsorbé en contact avec une sonde d'ADN de HTLV-III selon la revendication 6; et
(d) à déterminer si la sonde est hybridée avec l'acide nucléique adsorbé.

17. Lignée de cellules hybridomes qui produit un anticorps réagissant spécifiquement avec un polypeptide de HTLV-III selon la revendication 1.

18. Polypeptide selon la revendication 1, utilisable en thérapie, par exemple en vaccination.

19. Utilisation d'anticorps monoclonaux selon la revendication 10 pour la préparation d'un médicament utilisable en immunothérapie contre le syndrome d'immunodéficience acquise.

20. Utilisation d'un polypeptide selon la revendication 1 pour la préparation d'un médicament utilisable dans la vaccination contre le syndrome d'immunodéficience acquise.

Revendications pour l'Etat contractant suivant : AT

1. Procédé de préparation d'un polypeptide exprimé par des cellules transformées par un vecteur recombiné contenant l'ADN de HTLV-III, ledit polypeptide étant immunoréactif avec les sérums d'individus atteints du syndrome d'immunodéficience acquise ou avec des sérums contenant des anticorps anti-HTLV-III, ledit ADN de HTLV-III étant (a) un fragment de restriction de EcoRI d'environ 1,1 kilobases, comportant une séquence de nucléotides qui s'étend approximativement du nucléotide 4228 au nucléotide 5327 de l'ADN de HTLV-III tel que représenté sur la fig. 3, ou (b) un équivalent dudit ADN qui code pour un équivalent immunologiquement fonctionnel dudit polypeptide, comprenant les étapes consistant:
(i) à couper de l'ADN de HTLV-III pour produire des fragments d'ADN;
(ii) à insérer les fragments d'ADN dans un vecteur d'expression pour former un vecteur recombiné;
(iii) à transformer une cellule hôte appropriée avec le vecteur recombiné; et
(iv) à cultiver la cellule hôte transformée dans des conditions adéquates pour l'expression du polypeptide pour lequel code l'ADN de HTLV-III inséré.

2. Procédé selon la revendication 1, dans lequel l'étape de coupure consiste
(a) à mettre à digérer l'ADN de HTLV-III avec une endonucléase de restriction pour produire des fragments de restriction d'ADN, ou
(b) à couper l'ADN de HTLV-III pour produire des fragments d'ADN.

3. Procédé selon la revendication 1 ou 2, dans lequel le vecteur d'expression est pMR 100.

4. Essai immunologique pour la détection de HTLV-III, au moyen d'un anticorps, par exemple d'un anticorps monoclonal réagissant spécifiquement avec un polypeptide produit par le procédé selon la revendication 1.

5. Essai immunoradiométrique du type en sandwich pour la détection de HTLV-III, au moyen d'un anticorps immobilisé tel que défini dans la revendication 4 qui réagit avec le polypeptide

de HTLV-III, et d'un anticorps soluble tel que défini dans la revendication 4 qui réagit avec le polypeptide de HTLV-III.

6. Essai faisant intervenir l'utilisation d'un nécessaire comprenant un anticorps tel que défini dans la revendication 4 qui réagit spécifiquement avec le polypeptide de HTLV-III fixé sur une phase solide, et un anticorps soluble marqué tel que défini dans la revendication 4 qui réagit spécifiquement avec le polypeptide de HTLV-III.

7. Procédé in vitro de détection d'anticorps anti-HTLV-III dans un fluide corporel, comprenant les étapes consistant
(a) à mettre un immuno-adsorbant, comprenant un polypeptide selon la revendication 1 fixé sur une phase solide, en contact avec un fluide corporel jusqu'à de ce que les anticorps anti-polypeptide de HTLV-III contenus dans le fluide corporel fixent le polypeptide en phase solide;
(b) à séparer l'immuno-adsorbant du fluide corporel;
(c) à mettre l'immuno-adsorbant en contact avec un polypeptide marqué selon la revendication 1 ou un anticorps marqué anti-immunoglobuline humaine; et
(d) à déterminer la quantité de polypeptide marqué qui est fixée à l'immuno-adsorbant, en tant qu'indication de la présence de l'anticorps anti-HTLV-III.

8. Essai pour la détermination de la présence d'anticorps anti-HTLV-III dans un fluide corporel, faisant intervenir un nécessaire comprenant:
(a) un immuno-adsorbant comprenant le polypeptide de HTLV-III tel que défini dans la revendication 1, fixé sur une phase solide; et
(b) le polypeptide de HTLV-III marqué tel que défini dans la revendication 1 ou un anticorps marqué anti-immunoglobuline humaine.

9. Procédé de détection d'acide nucléique de HTLV-III dans un fluide corporel (par exemple un lysat de cellules), comprenant les étapes consistant:
(a) à adsorber sur un adsorbant l'acide nucléique contenu dans un fluide corporel;
(b) à dénaturer l'acide nucléique adsorbé;
(c) à mettre l'acide nucléique adsorbé en contact avec une sonde d'ADN de HTLV-III comprenant une séquence d'ADN essentiellement homologue de l'ADN tel que défini dans la revendication 1(a) ou (b); et
(d) à déterminer si la sonde est hybridée avec l'acide nucléique adsorbé.

10. Utilisation d'anticorps monoclonaux selon la revendication 4 pour la préparation d'un médicament utilisable en immunothérapie contre le syndrome d'immunodéficience acquise.

11. Utilisation d'un polypeptide selon la revendication 1 pour la préparation d'un médicament utilisable dans la vaccination contre le syndrome d'immunodéficience acquise.

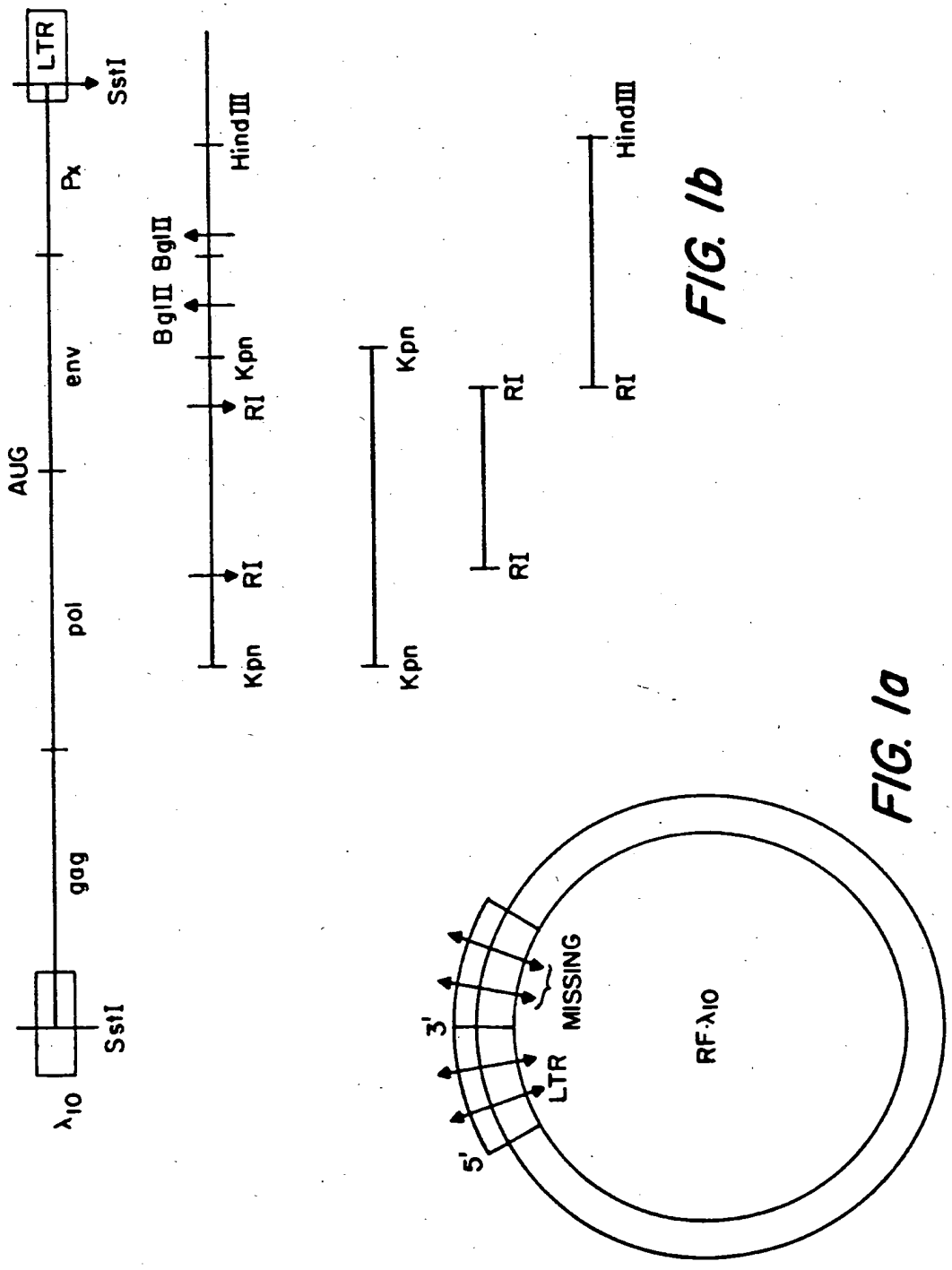
LTR
SstI
Px
env
AUG
pol
gag
SstI
λ10
BglII BglII
HindIII
RI Kpn
RI
Kpn
Kpn
Kpn
RI
RI
HindIII
RI
3'
5'
LTR
MISSING
RF·λ10

FIG. 1b

FIG. 1a

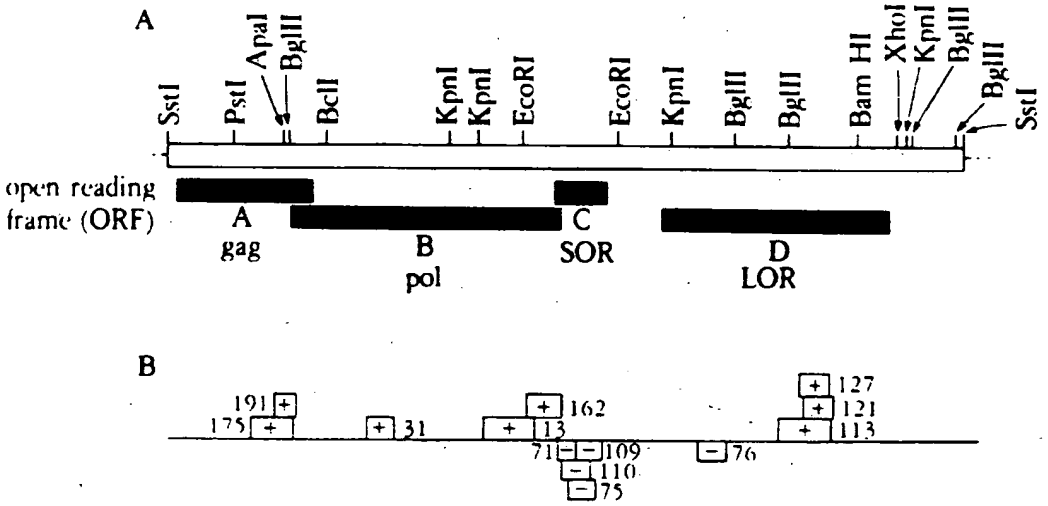
A
SstI
PstI
ApaI
BglII
BclI
KpnI
KpnI
EcoRI
EcoRI
KpnI
BglII
BglII
Bam HI
XhoI
KpnI
BglII
BglII
SstI
open reading frame (ORF)
A
gag
B
pol
C
SOR
D
LOR
B
191
175
31
162
13
71
109
110
75
76
127
121
113

FIG. 2

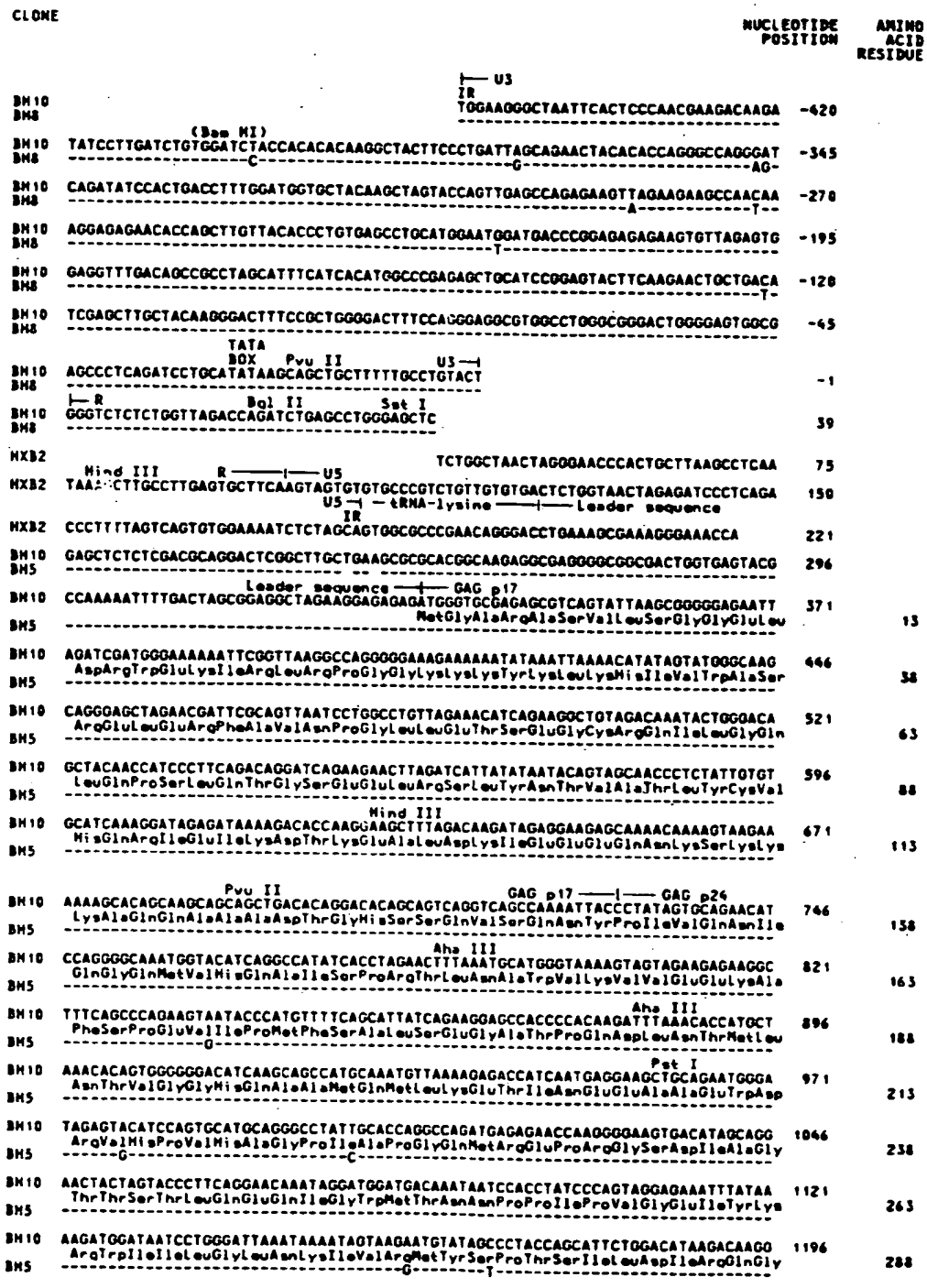
CLONE
NUCLEOTIDE POSITION
AMINO ACID RESIDUE
U3
IR
BH10
BH8
TGGAAGGGCTAATTCACTCCCAACGAAGACAAGA -420
(Bam HI)
TATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTAGCAGAACTACACACCAGGGCCAGGGAT -345
CAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGAGAAGTTAGAAGAAGCCAACAA -270
AGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCGGAGAGAGAAGTGTTAGAGTG -195
GAGGTTTGACAGCCGCCTAGCATTTCATCACATGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCTGACA -120
TCGAGCTTGCTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCG -45
TATA
BOX
Pvu II
U3
AGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTGTACT -1
R
Bgl II
Sst I
GGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTC 39
HXB2
TCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAA 75
Hind III
R
U5
TAAAACTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGA 150
U5
tRNA-lysine
Leader sequence
IR
CCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACCTGAAAGCGAAAGGGAAACCA 221
GAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACG 296
Leader sequence
GAG p17
CCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATT 371
MetGlyAlaArgAlaSerValLeuSerGlyGlyGluLeu 13
AGATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAG 446
AspArgTrpGluLysIleArgLeuArgProGlyGlyLysLysLysTyrLysLeuLysHisIleValTrpAlaSer 38
CAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACA 521
ArgGluLeuGluArgPheAlaValAsnProGlyLeuLeuGluThrSerGluGlyCysArgGlnIleLeuGlyGln 63
GCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGT 596
LeuGlnProSerLeuGlnThrGlySerGluGluLeuArgSerLeuTyrAsnThrValAlaThrLeuTyrCysVal 88
Hind III
GCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGAA 671
HisGlnArgIleGluIleLysAspThrLysGluAlaLeuAspLysIleGluGluGluGlnAsnLysSerLysLys 113
Pvu II
GAG p17
GAG p24
AAAAGCACAGCAAGCAGCAGCTGACACAGGACACAGCAGTCAGGTCAGCCAAAATTACCCTATAGTGCAGAACAT 746
LysAlaGlnGlnAlaAlaAlaAspThrGlyHisSerSerGlnValSerGlnAsnTyrProIleValGlnAsnIle 138
Aha III
CCAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGAAGGC 821
GlnGlyGlnMetValHisGlnAlaIleSerProArgThrLeuAsnAlaTrpValLysValValGluGluLysAla 163
Aha III
TTTCAGCCCAGAAGTAATACCCATGTTTTCAGCATTATCAGAAGGAGCCACCCCACAAGATTTAAACACCATGCT 896
PheSerProGluValIleProMetPheSerAlaLeuSerGluGlyAlaThrProGlnAspLeuAsnThrMetLeu 188
Pst I
AAACACAGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGA 971
AsnThrValGlyGlyHisGlnAlaAlaMetGlnMetLeuLysGluThrIleAsnGluGluAlaAlaGluTrpAsp 213
TAGAGTACATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGACATAGCAGG 1046
ArgValHisProValHisAlaGlyProIleAlaProGlyGlnMetArgGluProArgGlySerAspIleAlaGly 238
AACTACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCCAGTAGGAGAAATTTATAA 1121
ThrThrSerThrLeuGlnGluGlnIleGlyTrpMetThrAsnAsnProProIleProValGlyGluIleTyrLys 263
AAGATGGATAATCCTGGGATTAAATAAAATAGTAAGAATGTATAGCCCTACCAGCATTCTGGACATAAGACAAGG 1196
ArgTrpIleIleLeuGlyLeuAsnLysIleValArgMetTyrSerProThrSerIleLeuAspIleArgGlnGly 288

FIG. 3

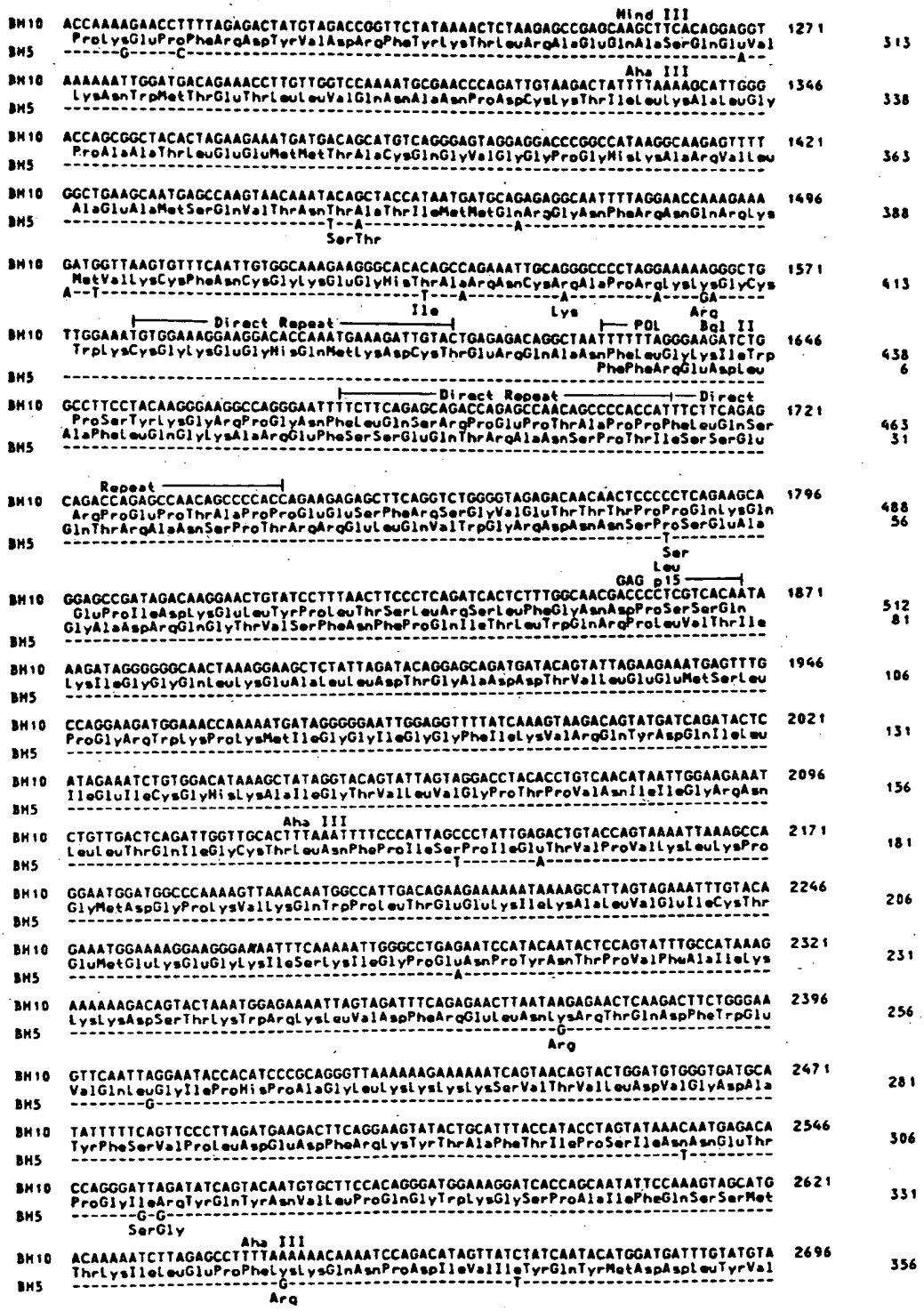

FIG. 3 (CONT.)

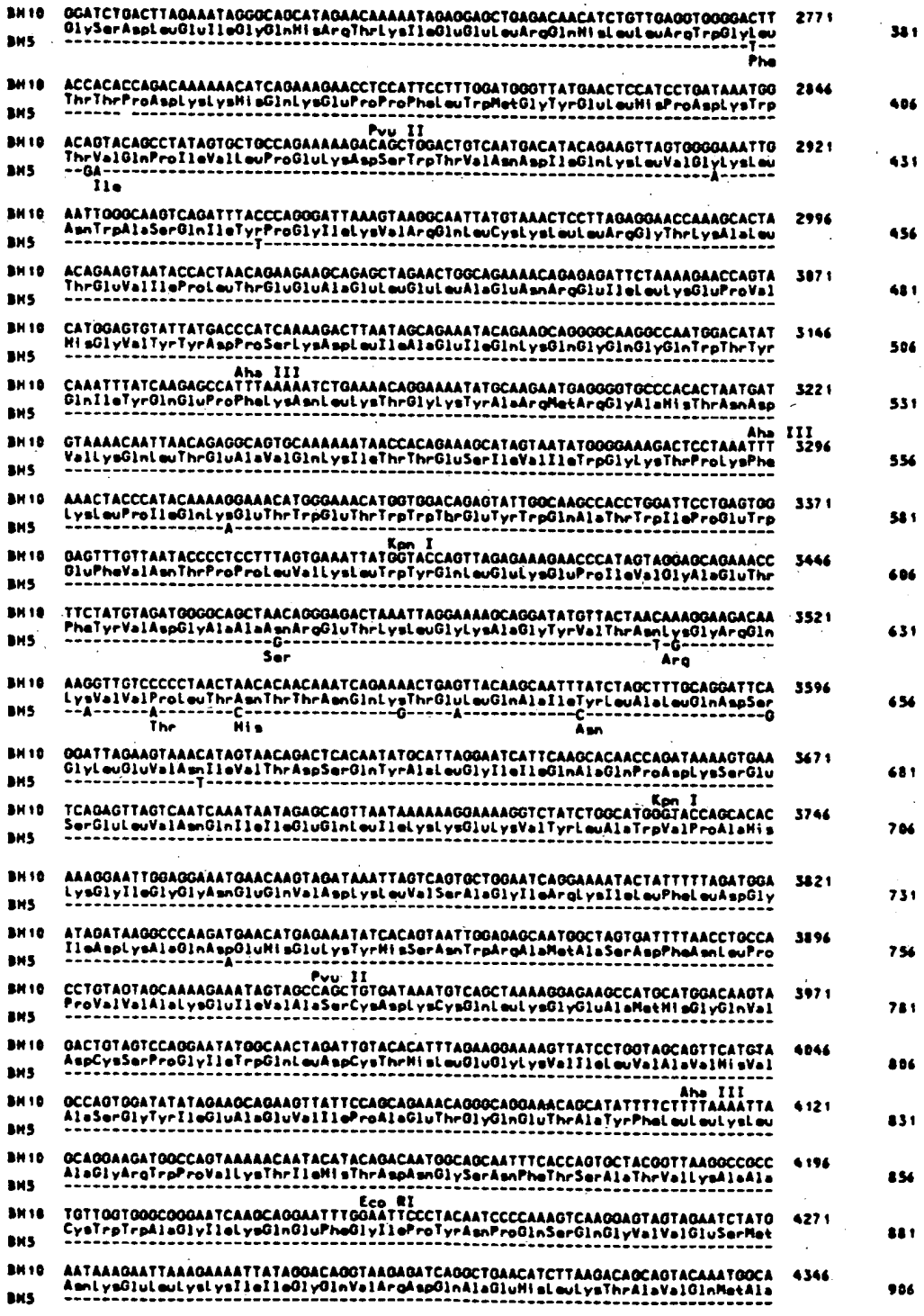

FIG. 3 (CONT.)

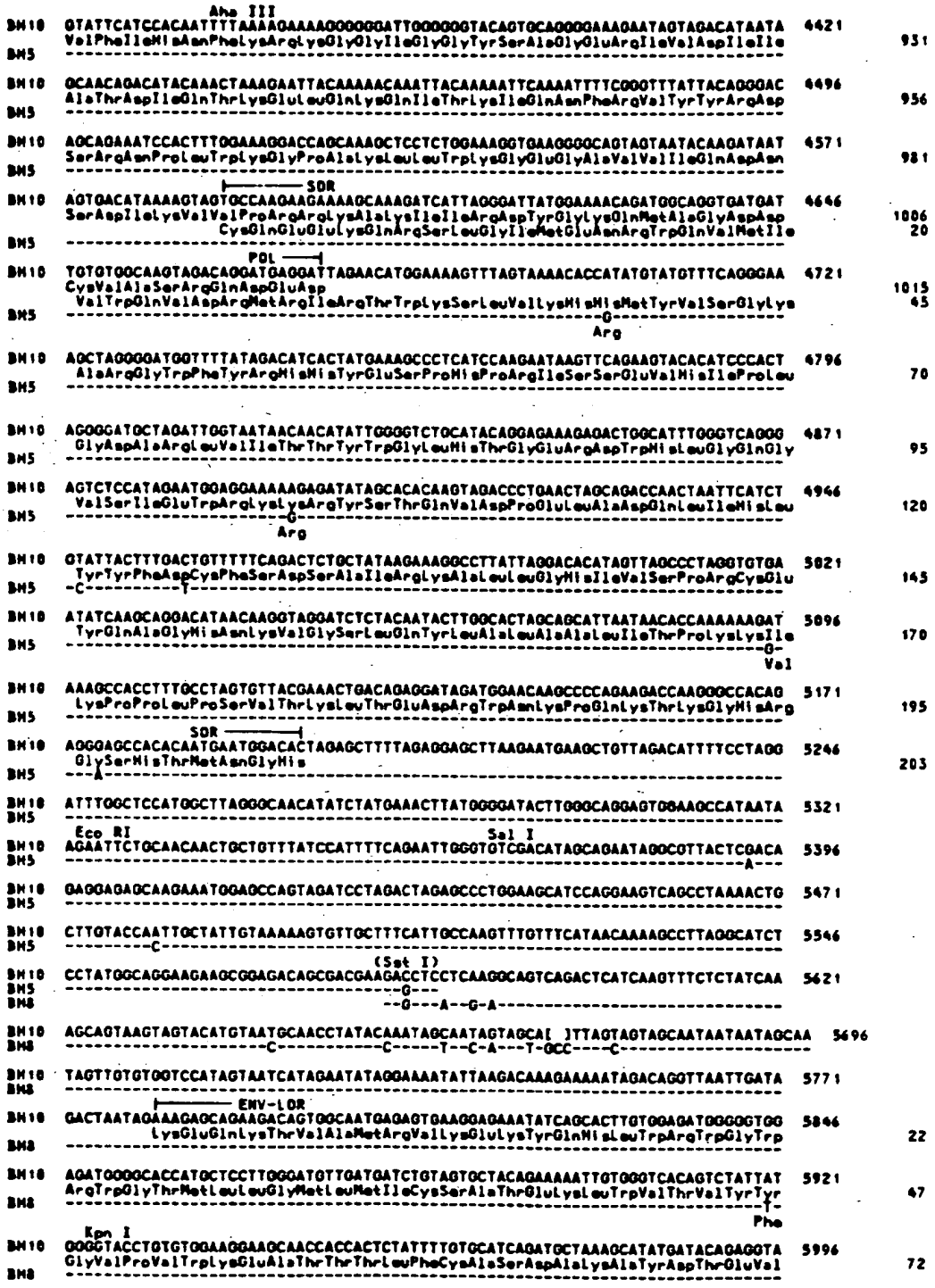

FIG. 3 (CONT.)

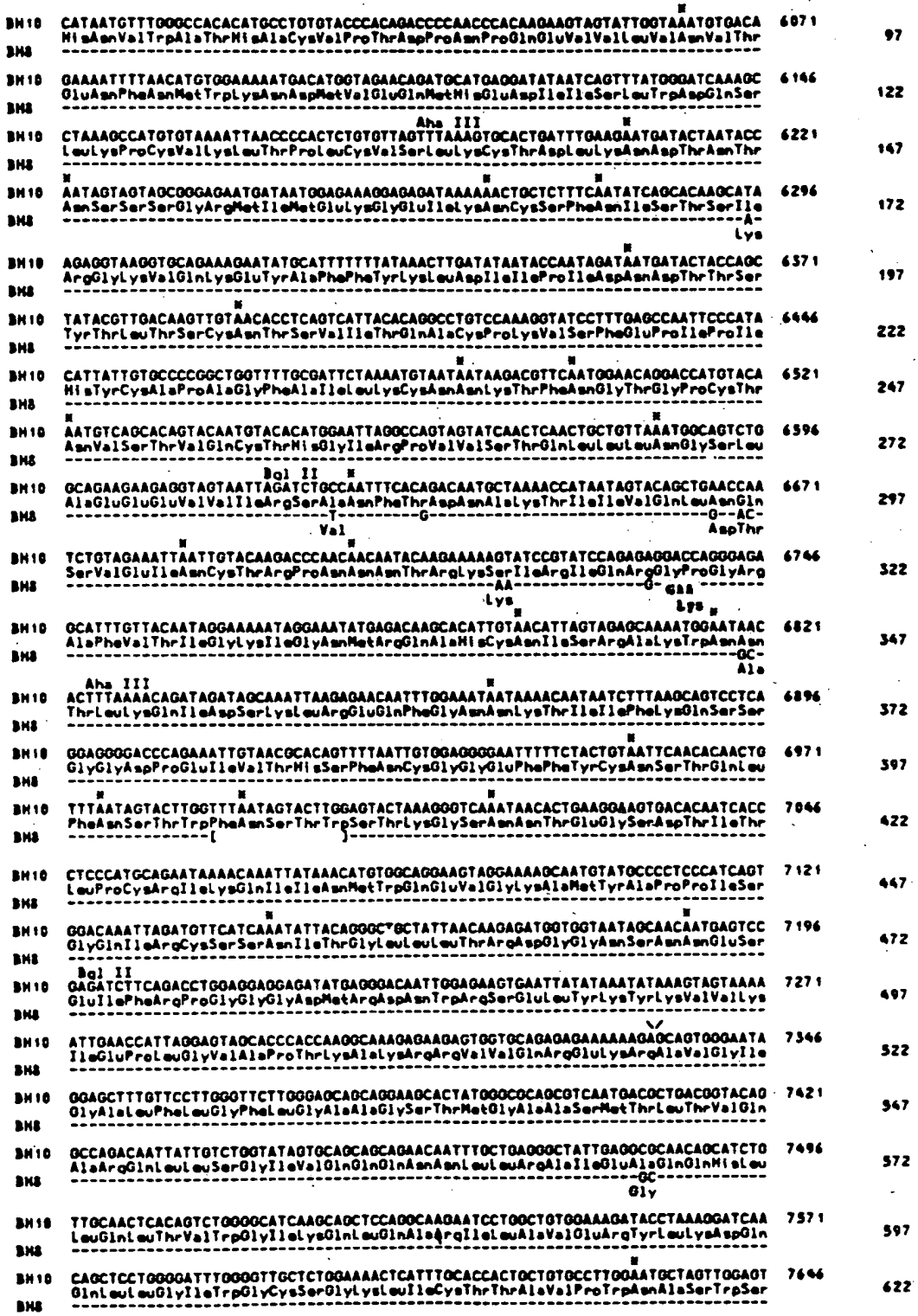

FIG. 3 (CONT.)

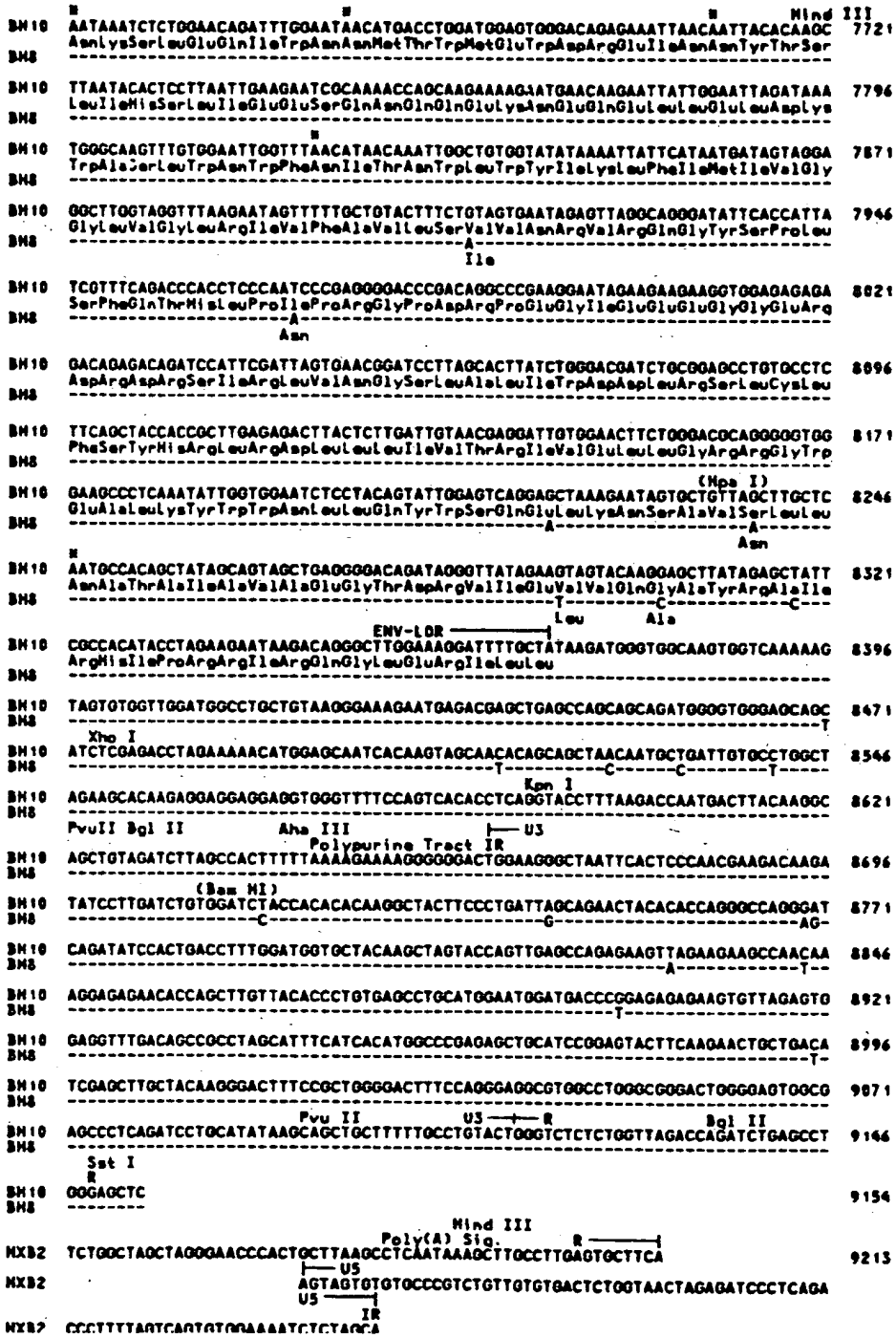

FIG. 3 (CONT.)

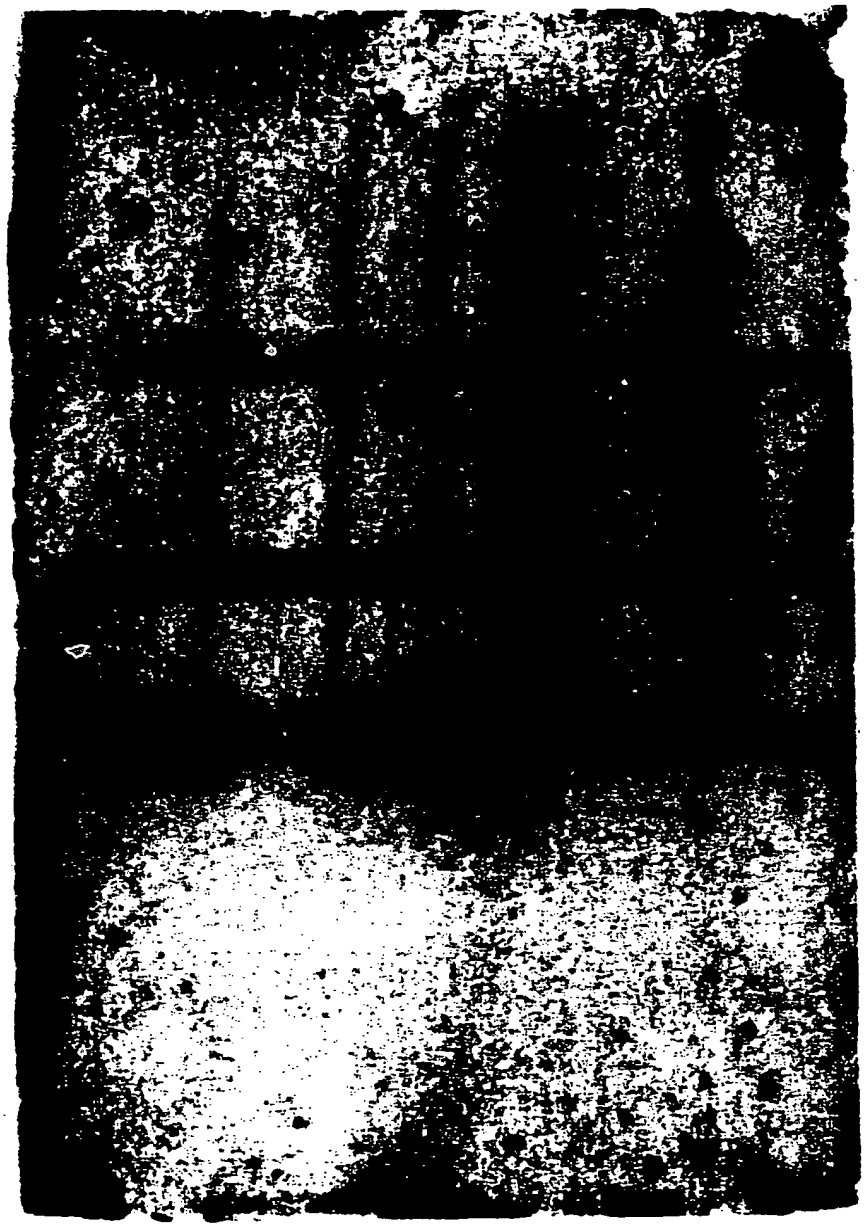

FIG. 4

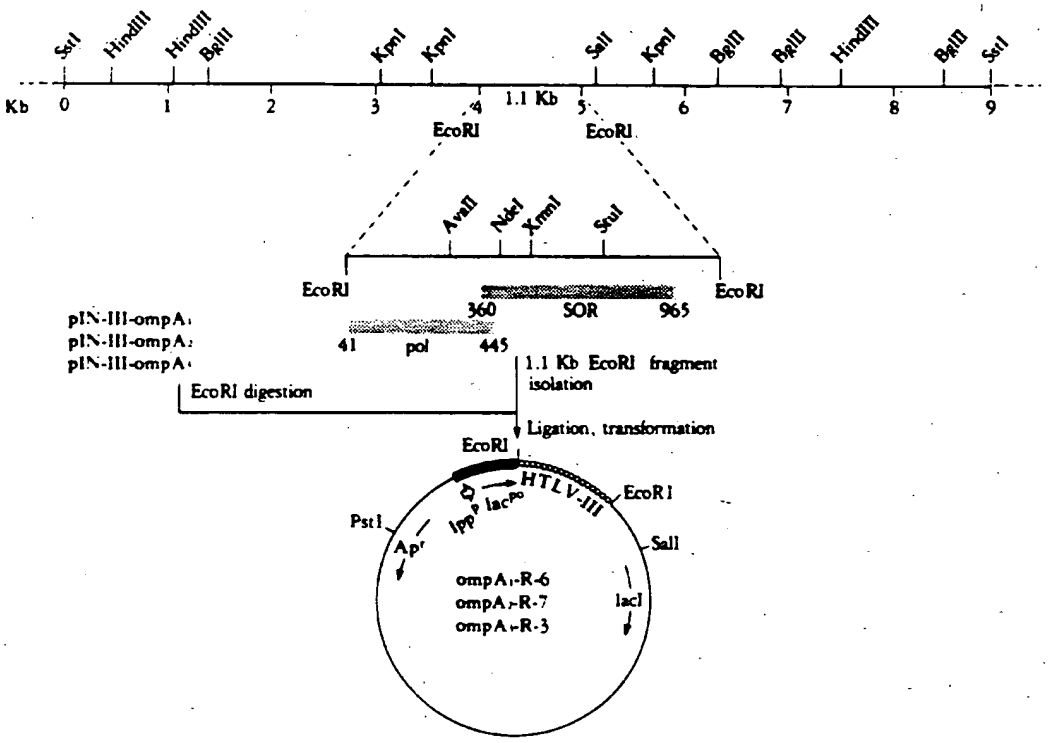
Sst I
HindIII
HindIII
BglII
KpnI
KpnI
SalI
KpnI
BglII
BglII
HindIII
BglII
Sst I
Kb
0
1
2
3
4
1.1 Kb
5
6
7
8
9
EcoRI
EcoRI
AvaII
NdeI
XmnI
SstI
EcoRI
360
SOR
965
EcoRI
pIN-III-ompA1
pIN-III-ompA2
pIN-III-ompA3
41
pol
445
1.1 Kb EcoRI fragment isolation
EcoRI digestion
Ligation, transformation
EcoRI
HTLV-III
EcoRI
PstI
Ap
Sall
ompA1-R-6
ompA2-R-7
ompA3-R-3
lacI

FIG. 5

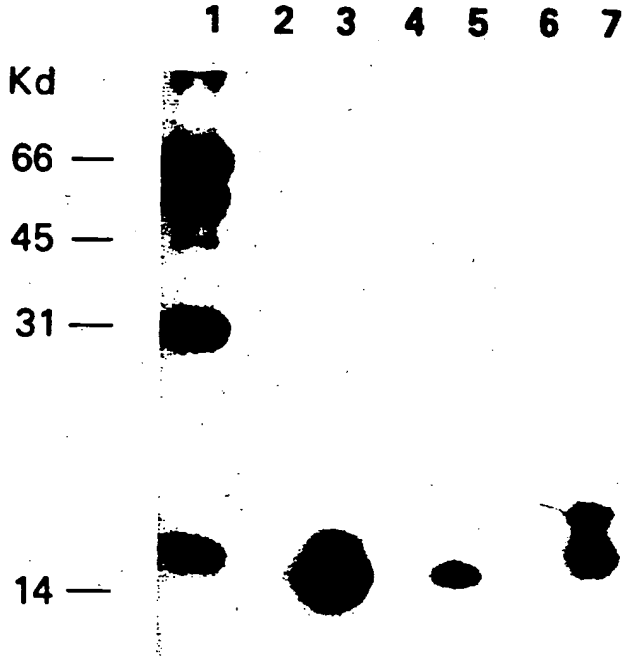

FIG. 6

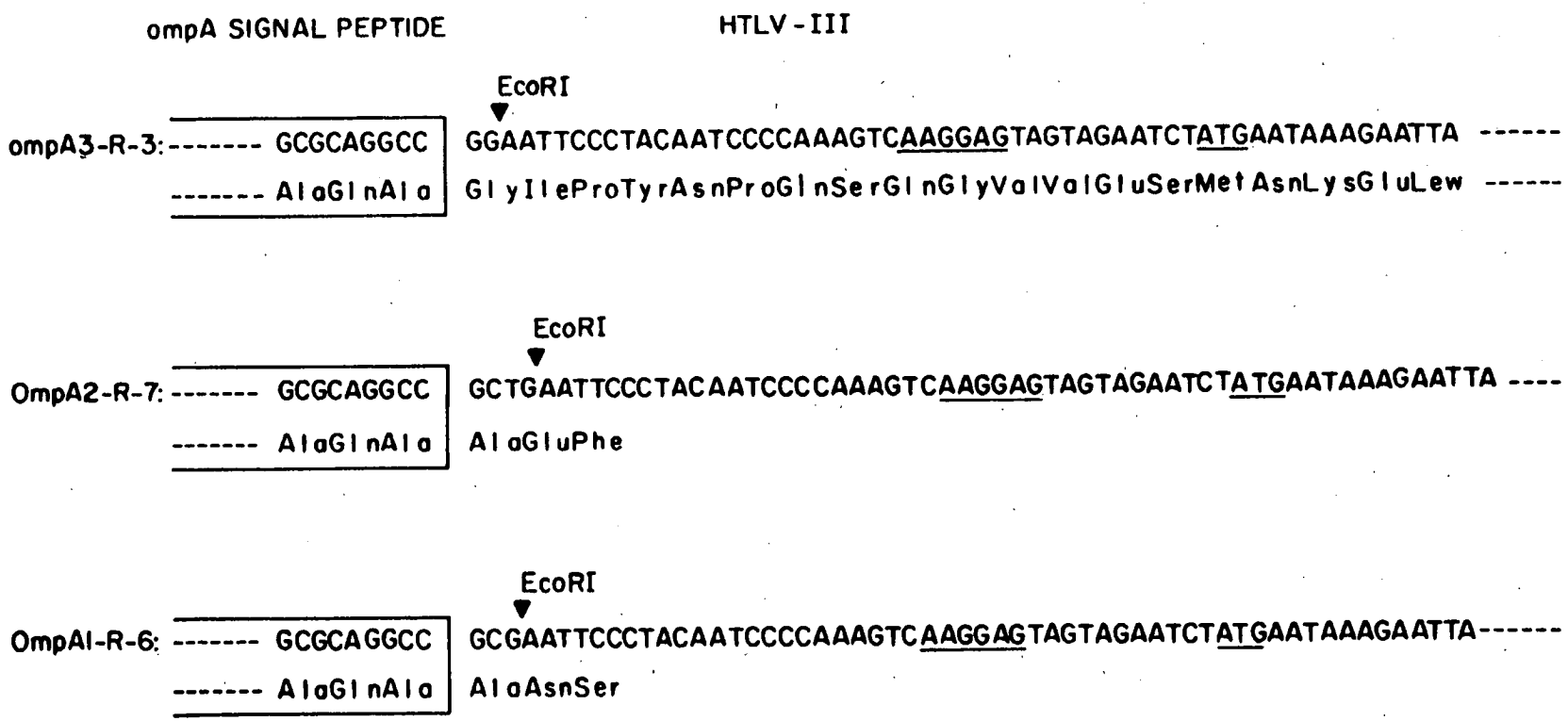
ompA SIGNAL PEPTIDE
HTLV-III
EcoRI
ompA3-R-3: ------- GCGCAGGCC
GGAATTCCCTACAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAAAGAATTA ------
------- AlaGlnAla
GlyIleProTyrAsnProGlnSerGlnGlyValValGluSerMetAsnLysGluLew ------
EcoRI
OmpA2-R-7: ------- GCGCAGGCC
GCTGAATTCCCTACAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAAAGAATTA ----
------- AlaGlnAla
AlaGluPhe
EcoRI
OmpA1-R-6: ------- GCGCAGGCC
GCGAATTCCCTACAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAAAGAATTA------
------- AlaGlnAla
AlaAsnSer

FIG. 6a

FIG.7

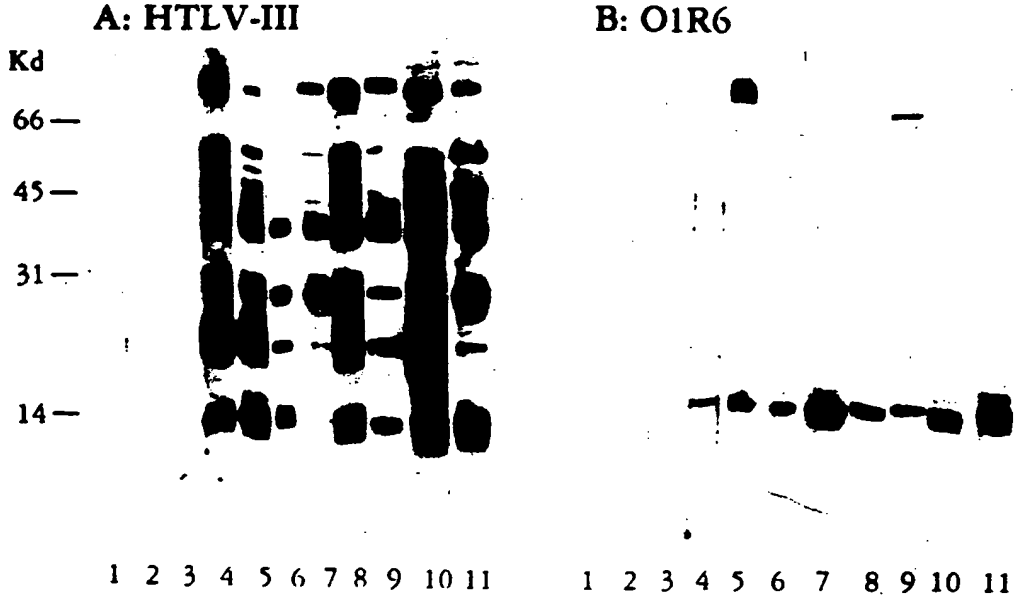

FIG.8

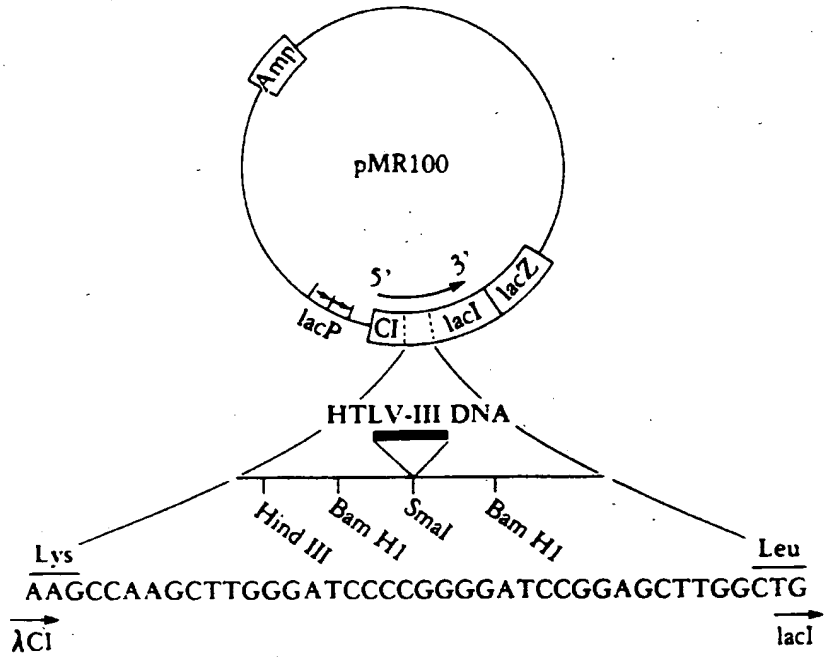
Amp
pMR100
5'
3'
lacP
CI
lacI
lacZ
HTLV-III DNA
Hind III
Bam H1
SmaI
Bam H1
Lys
Leu
AAGCCAAGCTTGGGATCCCCGGGGATCCGGAGCTTGGCTG
λCI
lacI

FIG. 9

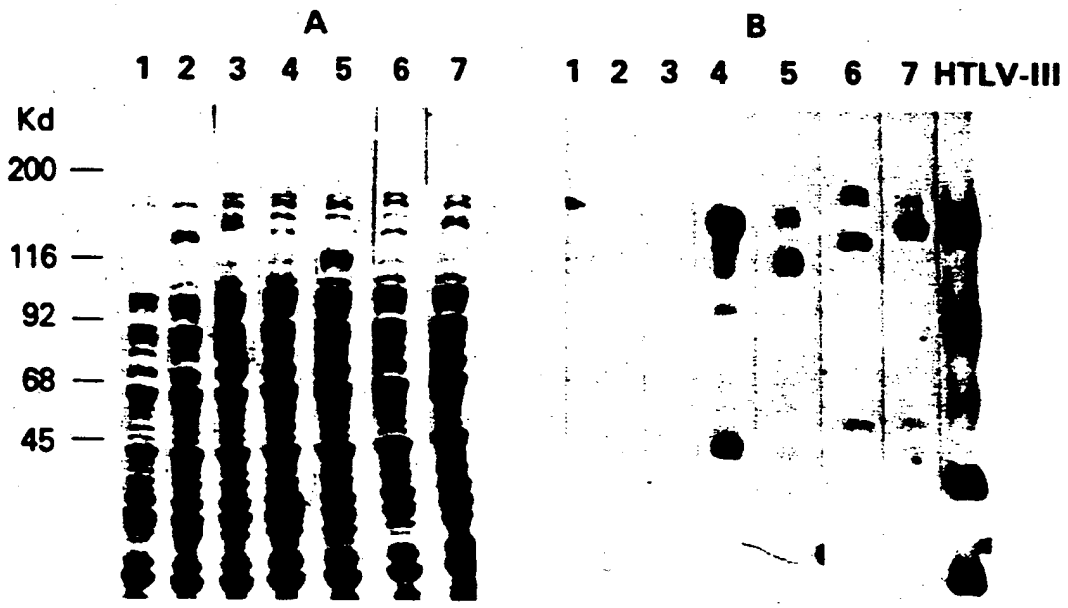

FIG. 10

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